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SEVENTEENTH ANNUAL MEETING.

PHILADELPHIA, PA., DECEMBER 27 and 28, 1904.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

A CONTRIBUTION TO CELL-CHEMISTRY.

By VICTOR C. VAUGHAN.

After the bacteria have reached maturity, they are removed from the agar in which they have grown, and washed with dilute alcohol, then extracted with absolute alcohol, and finally with ether. After this they are finely powdered and heated for from one to two hours in a flask with reflex-condenser with twenty-five times their weight of sodium alcoholate (2 per cent of sodium hydrate in absolute alcohol). This splits the cell-substance into two portions, which I will designate as *A* and *B*.

Portion *A* constitutes in round numbers about one-third of the cell-substance. It is freely soluble in water and absolute alcohol, insoluble in ether, petroleum ether, and chloroform. Its aqueous solutions are decidedly acid to litmus, and decompose sodium bicarbonate. It gives all the proteid color-reactions, and can be divided into sub-portions (*a* and *a'*) by precipitation with platinum chloride. Portion *a* constitutes from 10 to 15 per cent of *A*, is freely soluble in water and absolute alcohol, gives all the color-reactions for proteids, and kills animals (guinea-pigs, rabbits, and goats), in amounts varying with the animal and method of administration, in less than an hour. Its poisonous effects are identical with, but much more rapid than those of the living bacteria and the dead germ-substance. It lowers the temperature, and kills by arresting respiration. Animals treated with gradually increased doses of *a* acquire an active immunity to the living germ, and furnish a serum containing an antitoxin.

Therefore, portion *a* is the intracellular toxin of the bacillus.

Portion *B* is soluble in water, partly soluble in dilute alcohol, insoluble in absolute alcohol, and consists of several sub-portions

(*b*, *b'*, *b''*, etc.). When portion *B* is dissolved in water, and acidified with hydrochloric acid, portion *b* is precipitated; after removing this precipitate by filtration, and on heating the filtrate, portion *b'* is thrown down, while *b''* remains in solution. Portion *b'* is soluble in water, insoluble in absolute alcohol, thermostabile, and hæmolytic. In neutral or feebly acid solution, it dissolves red blood-cells at body-temperature after a period of incubation. Portion *b''* splits hæmoglobin with the formation of hæmatin. Animals treated with portion *B* acquire immunity to the living germ, and furnish a bacteriolytic, but not an antitoxic, serum.

The essential part of the germ-substance is a definite chemical compound, containing, along with other groups, toxic, hæmolytic, and hæmoglobin splitting groups.

Toxins have been extracted so far in my laboratory from colon, typhoid, and anthrax cells, and immunity has been secured with the isolated toxins of the first two.

THE HYDROLYTIC CLEAVAGE OF PROTOALBUMOSE.

By P. A. LEVENE.

The observation described in this report has been made in the course of a comparative study of the composition of various albumoses.

In the fraction containing the hexon bases of the protoalbumose a substance differing in its properties from the three known bases was observed. It did not readily form compounds with picric acid and platinic chloride. Towards silver solution it behaved similarly to histidin. The copper and the silver salts were analyzed. The analysis made it probable that the substance had the composition $C_{12}H_{22}N_4O_5$. (Substances with the composition of $C_{12}H_{24}N_2O_5$ and $C_{12}H_{26}N_2O_5$ have been described by Skraup and by Fischer respectively.)

For $C_{12}H_{20}N_4O_5Cu$

	Calculated.	Found.
C	39.67 per cent	40.14 per cent
H	5.51 "	5.39 "
N	15.45 "	16.40 "
O	22.04 "	22.77 "
Cu	17.35 "	15.30 "

The analysis of the silver salt gave the following results:

For $C_{12}H_{20}N_4O_5Ag_2$

	Calculated.	Found.
N =	10.89 per cent	10.32 per cent
Ag =	41.63 "	41.11 "

Further analysis of the substance is in progress.

CILIARY REVERSAL IN METAZOANS.

By G. H. PARKER.

The reversal of the effective stroke of cilia, though common among protozoans, has been recorded only in a few scattered cases among metazoans (sponges, sea-anemones, planarians, larvæ of marine worms, gills of mussels, and possibly frog embryos). In a common sea-anemone, Metridium, the labial cilia ordinarily strike outward and filter-paper pellets, etc., when placed on the lips, are discharged from the mouth. A piece of crab-meat placed on the lip causes the cilia to reverse, and it is thus swept inward. The cilia reverse to a filter-paper pellet soaked in crab-meat juice, but not to a piece of crab-meat deprived of most of its extractives. The crab-meat juice contains the chlorides of sodium, potassium, calcium, and magnesium. Sea-water containing about 2.5 per cent of potassium chloride will reverse the cilia; the other chlorides will not. The labial cilia will live several hours in $\frac{5}{8}\,m$ NaCl solution. They reverse in a $\frac{5}{8}\,m$ NaCl + $\frac{1}{3}\,m$ KCl solution which has an osmotic pressure of about 36.9 atmospheres, but not in a $\frac{5}{8}\,m$ NaCl + $\frac{1}{3}\,m$ NaCl solution which has an osmotic pressure of about 36.0 atmospheres. Hence, reversal is not produced by osmotic pressure nor by Cl ions. They reverse in $\frac{5}{8}\,m$ NaCl + $\frac{1}{3}\,m$ KNO_3, but not in $\frac{5}{8}\,m$ NaCl + $\frac{1}{3}\,m$ $NaNO_3$. Hence, reversal is due to K ions. Meat-juice containing much less potassium than the weakest artificial mixture producing reversal will still cause a vigorous reversal. Hence the reversal caused by meat-juice is probably not simply dependent upon the contained potassium. Temporary reversals of this kind may occur in the ciliated organs of many of the higher animals, for instance, the palps of lamellibranchs, and the female genital ducts of vertebrates.

A STUDY OF THE CONDITIONS FOLLOWING THE ESTABLISHMENT OF THE ECK FISTULA IN DOGS.

By P. B. HAWK.

The operations were successfully performed by Dr. J. E. Sweet. The liver function was greatly impaired.

Dog No. 2. Body-weight, 9.64 kg. Fed mixed diet eleven days, and on the following four days beef meal (pre-digested powdered beef) and milk. On the afternoon of the fourth day of this diet there were pronounced ataxia, entire loss of sight and hearing, complete anæsthesia and catalepsy. In about four hours the symptoms began to abate, and on the next day had entirely disappeared. The animal fasted for twenty hours, and was then placed on a diet of fresh lean beef. On the morning of the fifth day of this diet symptoms were observed similar to those occurring after the beef-meal diet. These disappeared in about five hours, leaving the dog very weak. The animal had now lost 28 per cent body-weight. Placed on a diet of milk and bread the animal died on the fifty-ninth day of the experiment. A poor appetite and a large loss in body-weight (42 per cent) were noted. The autopsy revealed about 800 c.c. of ascitic fluid in the body-cavity, and numerous whitish nodules the size of a small pea in the omentum, mesentery, and liver, smears from which showed presence of numerous tubercle bacilli. The fistula was about 2 cm. long. There were no anastomoses.

Dog No. 4. Body-weight, 13.08 kg. Beef fed thirty days with negative result. On thirty-first day injected sodium carbamate. No effect. Beef fed twenty-nine days, and no symptoms resulted. Liebig's extract was added to the diet, and on the morning of the tenth day of this diet, and seventieth of the experiment, symptoms were noted similar to those observed in the case of Dog No. 2, in this instance being supplemented by tetanus. There was a loss of 15 per cent in body-weight. The autopsy showed no anastomoses and no tubercular nodules. The fistula was 2 cm. long.

The feeding of sodium carbamate, and the injection of this salt into the blood-stream of normal dogs, were productive of none of the symptoms noted above.

ON JENSEN'S THEORY OF GEOTROPISM IN PARAMŒCIUM.

By E. P. LYON.

JENSEN[1] attempts to explain the orientation of paramœcium in response to gravity by supposing that the difference in hydrostatic pressure between the upper and lower surfaces of the animal is the effective stimulus. But this difference is extremely small. Assuming that the organism is at a depth of ten centimetres in water, that the pressure of the atmosphere is 1030 grams per square centimetre, and that the thickness of the animal is 0.001 cm., I calculate that, according to Jensen's theory, the protozoan would be capable of responding to a pressure-stimulus of only $\frac{1}{1,040,000}$ of the total pressure on one side, or a still smaller fraction of the total pressure on the entire cell.[2]

Moreover, when one increases the total pressure, the difference in pressure between the upper and lower surfaces being due to the difference in depth must remain constant. If Weber's law holds good, one should be able to reach a total pressure of such magnitude that the organism would no longer respond to the very small difference in pressure between its upper and lower sides. I have increased the pressure up to three atmospheres, and do not find a diminished response. On the other hand, if one removes the whole or part of the atmospheric pressure, thus reducing the total pressure to a small fraction of its former intensity, one would expect the stimulus (which is now a much larger fraction of the total pressure), to produce quicker and more accurate orientation. Such is not the case.

Using the centrifugal machine in the ordinary way, *i. e.*, with the tubes open towards the axis of rotation, one can increase the acceleration (equivalent to gravity), with but very slight or no increase of total pressure. If the centrifugal force be greater than gravity (within limits) the response of the animals is quickened, even though the pressure be unchanged.

Finally, if one uses on the centrifuge tubes open at the outer end and closed at the axis, the condition is such that the pressure in-

[1] JENSEN: Archiv für die gesammte Physiologie, 1893, liii, p. 428.

[2] When this was written I had not seen JENNINGS' last paper in which practically the same calculation is made, and JENSEN'S theory rendered doubtful by quite different observations from my own. See JENNINGS: Journal comparative neurology and psychology, 1904, xiv, p. 442.

creases from the periphery toward the axis. Nevertheless, the animals go against the centrifugal force, and from low to high pressure. Therefore Jensen's theory is incorrect.

ON PARA-LACTIC ACID.[1]

By ARTHUR R. MANDEL (read by invitation).

Experiments by Ray, McDermott, and Lusk[2] showed that after producing phlorhizin diabetes in a fasting dog poisoned with phosphorus, the urine changed from an ammoniacal to an acid reaction. It seemed possible that lactic acid, which is produced in phosphorus-poisoning, and probably is the cause of the excessive quantity of ammonia in the urine, might be derived as a cleavage product of sugar which originates from proteid. If this be true, no lactic acid should be formed in diabetes even though phosphorus-poisoning be present.

Experiments showed that such was the case. The blood and urine of a fasting dog, poisoned with phosphorus, contained lactic acid, but this disappeared when diabetes was induced by phlorhizin. In another case, diabetes was induced, and then for three days phosphorus and phlorhizin were administered. At the end of the period there was no lactic acid in the blood or in the urine.

It seems, therefore, probable that the lactic acid in phosphorus-poisoning is derived from the cleavage of sugar.

Preliminary experiments after ingesting fermentation lactic acid in diabetes apparently show a slight reduction of proteid metabolism, and therefore of sugar output, and also indicate the synthesis of a small quantity of lactic acid into sugar.

FURTHER RESULTS IN SUPRARENAL GRAFTING.

By F. C. BUSCH and C. VAN BERGEN.

Five cases of suprarenal grafting in rabbits' kidneys, in addition to the one reported at the last meeting of the society, have shown physiological activity of the grafts.

In all of these cases, living grafts, composed in whole or in part of

[1] Read by Dr. Graham Lusk.

[2] Ray, W. E., T. S. McDermott, and G. Lusk: This journal: 1899, iii, p. 139.

the medullary portion of the suprarenal, have been demonstrated microscopically, from thirty-one to two hundred and forty-seven days after transplantation.

A METHOD FOR THE QUANTITATIVE ESTIMATION OF CARBAMATES IN ANIMAL FLUIDS.

By J. J. R. MACLEOD and H. D. HASKINS.

Although the method described by us in this journal (Vol. XII, p. 444) for the estimation of carbamates in blood gives very accurate results for added carbamate, we do not consider it, as yet, sufficiently reliable for the *detection* of minute quantities of carbamate in blood. Thus we have, on several occasions, obtained a slight positive reading in the unheated tube (B), which certainly did not come from carbamate. When the blood is in the slightest laked, this positive reading is invariably obtained. Whenever possible, we use as the control, instead of the heated specimen, some normal blood-serum treated exactly like that under examination.

We have made a number of estimations of the rate of decomposition of carbamate of ammonia in blood and in water, and have found that at room-temperature blood-serum retards the decomposition to a slight extent. Thus, carbamate of ammonia, yielding 0.950 c.c. of gas, was dissolved in blood-serum; after half an hour 8 per cent of this gas was recovered. In water in which this amount of carbamate was dissolved only the minutest trace of gas due to carbamate could be obtained. This preserving property would appear to be due to the inorganic salts, for artificial plasma yields a similar result.

SOME REMARKS ON THE CHEMISTRY OF CARBAMATES.

By J. J. R. MACLEOD and H. D. HASKINS.

When ammonia water is added to a solution of sodium carbonate or bicarbonate no carbamate is formed unless there be a large excess of ammonia present. Thus, when solutions of ammonia and of sodium carbonate were mixed in such quantities as to satisfy the equation $2\ NH_4OH + Na_2CO_3 = (NH_4)_2CO_3 + 2\ NaOH$, and allowed to stand in a well-stoppered vessel over night, no trace of carbamate could be

detected by the method described by us in this journal (Vol. XII, p. 444), or by Drechsel's method. In the presence of a large excess of ammonia however (1 c.c. $\frac{n}{1}$ Na_2CO_3 + 8 c.c. $\frac{n}{1}$ NH_4HO) carbamate was formed in small amount (Drechsel's method).[1]

By mixing solutions of ammonium chloride and sodium carbonate in various proportions, large amounts of carbamate were invariably obtained.

These results conclusively demonstrate that carbamates are not readily formed when preformed ammonium hydroxide is brought in contact with soluble carbonates; whereas ammonia (NH_3) in the free state ("im Enstehungszustande") readily forms it. Drechsel's conclusion that carbamate can result without the ammonia being free, would therefore not hold unless when a large excess of ammonia is present.

It appears to us that this result satisfactorily explains why no carbamate should be formed when a mixture of ammonium chloride and milk of lime is treated with sodium carbonate solution, whereas it is formed when the sodium carbonate is added before the milk of lime.[2]

When we first noted this result, we thought that the presence of the sodium hydroxide formed by the chemical reaction might be the cause of the non-formation of the carbamate. That such is not the case we have shown by the fact that carbamate readily forms in a mixture of 5 c.c. $\frac{n}{1}$ Na_2CO_3, 5 c.c. $\frac{n}{1}$ NH_4Cl, and 10 c.c. $\frac{n}{1}$ NaOH.

In the oxidation at body-temperature of glycocoll, aspartic acid, and tartaric acid, with ammonium permanganate in the presence of 2.5 per cent ammonia water (*i. e.*, 0.7 per cent NH_3), a very large amount of carbamate is produced, especially at an early stage in the process. In our experiments, total CO_2 and carbamate CO_2 were estimated at varying intervals during the oxidation, the permanganate being added in small quantities at a time (0.05 gm.). The following were the maximal values, expressed as per cent of carbamate to total CO_2: glycin 25 per cent, aspartic acid 43 per cent, and tartaric acid 29 per cent. With oxalic acid at first, acetamid and ethyl alcohol oxidation to CO_2 was very much slower, and only in the case of oxalic acid was any measurable amount of carbamate CO_2 obtained.[3]

[1] Drechsel: Journal für praktische Chemie, 1877, xvi, p. 180.

[2] Drechsel's first two experiments, *Loc. cit.*

[3] Hofmeister: Archiv für experimentelle Pathologie und Pharmakologie, 1896, xxxvii, p. 426.

ON THE ORIGIN OF KREATININ.

By WALDEMAR KOCH.

In the course of an investigation of the food-value and intermediary metabolism of lecithin, the interesting relation of its methyl groups to the methyl group of kreatinin suggested the possibility of measuring the amount of lecithin metabolism by the amount of kreatinin excretion. Feeding experiments with kreatin-free diets varying in lecithin from 0.5 to 7 gm. per day indicate that the amount of kreatinin can be varied, but only to a limited extent, as the capacity of the animal body for forming kreatinin seems to be limited. The excess of lecithin is probably stored, as it does not appear in the fæces. The figures which will be published later further indicate that besides the lecithin another factor (proteid) is involved in supplying most or all of the nitrogen of the kreatinin molecule. The possibility here suggested of two factors entering into the formation of kreatinin may lead to an explanation of the hitherto conflicting statements as to the source of this interesting substance.

ON THE ELIMINATION OF CREATININE.

By LAFAYETTE B. MENDEL and OLIVER E. CLOSSON.

The available data regarding the origin and elimination of creatinine in man are relatively meagre and conflicting. Thus the presence of creatinine in the urine of vegetarians has recently been denied by Caspari and Glæssner, while other observers have reported figures scarcely smaller than those noted in persons living on a mixed diet. The relation of creatinine excretion to various types of diet is rather uncertain ; and its variations under different conditions of nutrition are practically undetermined. The importance of an investigation of this subject is apparent in view of the fact that creatinine, next to urea, is one of the most prominent nitrogenous constituents of the urine, and its connection with muscular metabolism or proteid transformations may be significant.

The writers have determined the total creatinine output in a considerable number of individuals under varying conditions of diet, in health and disease. The collected statistics will be published later;

attention is here directed to the noteworthy excretion of creatinine in vegetarians, as well as in individuals living on a low proteid diet for long periods, and to the rate of excretion during brief intervals in comparison with the simultaneous total nitrogen and uric acid output. In general, on a creatin-free diet, there is a tendency towards parallelism between the total nitrogen output and the elimination of the creatinine group.

A CASE OF TUMOR OF THE FLOOR OF THE FOURTH VENTRICLE WITH CEREBELLAR SYMPTOMS, IN A CAT.

By FRANK P. KNOWLTON (read by invitation).

A CAT brought to the laboratory presented the following interesting condition:

There was a marked weakness of the muscles of the left side, as compared with those of the right. This weakness affected the hind limbs more than the fore limbs.

Spasm and rigidity of the muscles of the right side were especially noticeable, and there was a tendency toward extensor rigidity of the fore limbs.

The animal's body was curved to the left. The occiput was drawn to the left and the face to the right. The head and tail were bent to the left.

Tremor was present, increased by movement and by excitation.

Movement was in circles toward the left, and there was a tendency to roll around the long axis, and always to the left.

The animal was readily fatigued by slight exertion.

Examination of the eyes showed rotary mystagmus.

Hearing and cutaneous sensation seemed unimpaired, and there was no evidence of pain.

Post-mortem examination showed a tumor arising from the left half of the floor of the fourth ventricle and extending from the median line to the restiform body, at the level of the ventral nucleus of the cochlear nerve.

Microscopical examination of the specimen by Dr. H. S. Steensland showed that the tumor was a glioma.

Sections of the medulla and pons, stained by the Marchi method,

showed marked degeneration of the pons fibres and the eighth nerve on the left side, with compression and some degeneration in other tracts.

ON THE PRESENCE OF SOAPS IN THE ORGANISM IN CERTAIN PATHOLOGICAL CONDITIONS (A PRELIMINARY COMMUNICATION).

By OSKAR KLOTZ (by invitation).

This I can quite confirm, that the centre of the calcareous deposits consists of calcium salts laid down in a homogeneous matrix (Litten), but this is not the case at the periphery, where the active process of calcification is taking place. On the contrary, here we find substances which chemically react as soaps, and can be isolated as such. Such soaps are here present as the potassium, sodium, or even the ammonium compounds of the fatty acids, being rendered insoluble by the combination with albumins of the degenerating tissues. Such soap-albumin compounds, in the presence of the calcium of the blood, form insoluble calcium curds or double calcium soaps. These insoluble calcium substances are then decomposed into the phosphate and carbonate of lime, which, as has been said, remain as the permanent deposits of calcium in a homogeneous matrix. It is further shown that soaps may be demonstrated in pus, particularly that of chronic abscesses, and Langerhaus has shown that a calcium soap is formed in fat necrosis.

In the production of an experimental calcareous degeneration in the kidneys of rabbits, it is shown by means of staining that the fatty changes taking place in the cells of the tubules is in the same location as the calcareous deposit, and further that in these kidneys a soap may be demonstrated by proper chemical analysis.

The following conclusions are arrived at:

1. The earliest change in cells, which later undergo calcareous degeneration, is one of cloudy swelling or coagulation necrosis.

2. Following this, fatty changes are noticeable in the cells, and now, by means of proper reagents, soaps with potassium, sodium, and presumably ammonium bases, can be detected.

3. Such soaps and albumins form a combination which is insoluble in water or salt solution.

4. Soaps and fatty acids have an affinity for the calcium salts in solution in the body-fluids, and form with them an insoluble compound.

5. Later, judging from the fact that phosphate and carbonate of lime are formed, and the deposits give no reaction for fats, the fatty acid moiety of the calcium soap is replaced by the more powerful carbonic and phosphoric acids.

OBSERVATIONS ON THE ALIMENTARY CANAL AFTER SPLANCHNIC AND VAGUS SECTION.

By W. B. CANNON.

In some animals the greater and lesser splanchnic nerves were cut on both sides; in other animals the right vagus nerve was cut below the recurrent laryngeal branch, and later the left vagus was divided in the neck. X-ray observations were made on the movements of food mixed with bismuth subnitrate. The prolonged stasis of food in the thoracic œsophagus after vagus section (noted first by Reid in 1839) is in marked contrast to the almost normal advance of the food after it has entered the stomach. The movements of the stomach and the intestines (peristalsis and rhythmic segmentation) were seen after vagus or splanchnic section. The stomach movements were inhibited by distress in both conditions. The difference in the rate of discharge of carbohydrates and proteids was also preserved in both conditions, but the discharge of both foodstuffs from the stomach was slower than normal when the vagi were cut. There was also a slight delay in the passage of the food through the small intestine after vagus section.

THE EFFECT OF CEREBRAL INJURIES ON THE BULBAR VASOMOTOR CENTRE.

By W. T. PORTER and T. A. STOREY.

The condition of the vasomotor centre was determined by measuring, with a membrane manometer, the fall in carotid blood-pressure following stimulation of the depressor nerve before and after injuries to the brain. Rabbits were used. They were anæsthetized, when

this was necessary to prevent pain. The experimental injuries consisted of removal of the cerebral hemispheres and cerebellum, fractures with intracranial hemorrhage following blows on the cranium, and compression of the brain by means of thin bags inserted through a trephine opening and distended with water.

The most extensive injuries did not inhibit the vasomotor centres. Indeed, the fall in blood-pressure following stimulation of the depressor nerve is commonly greater after the removal of the cerebral hemispheres than in the normal animal.

We are making similar experiments upon the cat and dog. As the depressor is not a separate nerve in the dog, and is frequently not separate in the cat, it is necessary to use in its place some other nerve afferent to the vasomotor centre. Professor Porter is determining to what extent the sciatic and other nerves thus replace the depressor nerve. Observations made simultaneously on the depressor and sciatic nerves, during shock produced by painting the intestines with nitric acid (in anæsthesia), show that the sciatic nerve may be safely employed to measure the working power of the vasomotor cells, although the necessary use of curare introduces some difficulties.

THE CURVE OF LESSENING CONDUCTIVITY DURING INCREASING TONUS OF THE HEART.

By W. T. PORTER and F. H. LAMB.

In 1893 it was suggested[1] that "fibrillar contractions of the heart may be due to an interruption of the contraction-wave. The contraction-wave would thus be prevented from running its usual course, and the normal co-ordinated action of the ventricular cells would give place to the confusion conspicuous in fibrillary contractions." In 1899 it was pointed out[2] that "the tonus of the ventricle is greatly increased at the onset of fibrillation, and that co-ordinated beats do not return unless the extreme, apparently tonic contraction is considerably lessened. The continued shortening observed may be due to an abnormal, long-continued, tonic contraction of a number of the cardiac fibres. The appearance of such a spasm at several points

[1] Porter, W. T.: Journal of physiology, 1893, xv, p. 134.
[2] Porter, W. T.: This journal, 1899, ii, p. 129.

might break up the normal conduction of the contraction wave by interposing here and there regions, the intense spasmodic contractions of which would block the passage of the contraction-wave, just as it is blocked experimentally by squeezing the muscle-fibres together with a clamp, and would leave the remaining muscle-fibres dissociated and in confusion." In 1902,[1] this hypothesis was brought formally to the attention of the Society. In 1903,[2] it was experimentally demonstrated that as the tonus increases the conductivity of the heart diminishes.

The present observations attempt to fix quantitatively the relation between the rise of tonus and the consequent fall in conductivity. This relation is expressed by a curve in which the millimetres of tonic shortening of the muscle are plotted as abscissæ, and the hundredth seconds of increase in latent period as ordinates. Such a curve steadily rises, but the rise is broken by deviations statistically accidental. By increasing the number of observations, these accidental errors may be balanced against each other, and thus eliminated. We have obtained in this way a curve which rises apparently in a straight line, as does the curve of developing energy in muscle.

We have also noted instances of extreme tonic spasm.

These observations support the hypothesis outlined above.

THE VOLUME-CURVE OF THE MAMMALIAN VENTRICLE (ILLUSTRATED).

By YANDELL HENDERSON and MARVIN McCRAE SCARBOROUGH.

The principal method employed was to enclose the ventricles (of dogs) in a plethysmograph, and to record the volume-changes by means of air-transmission and a tambour. Above the volume-curve was recorded the intraventricular or aortic pressure-curve.

The volume-curve of the ventricle, both in the heart-model shown at the last annual meeting of this society and in the living animal, closely resembles a series of inverted isotonic muscle-curves, and indicates that in the instant after systole is at an end, and the tension of the muscular walls is relaxed, the ventricles are refilled to their full

[1] Porter, W. T.: This journal, 1902, vi, p. xxiv.

[2] Porter, W. T.: This journal, 1903, viii, p. xxvi.

capacity by the rush of blood from the large veins and auricles. The ventricles are therefore full, and, as will be shown in another paper, the auriculo-ventricular valves are closed several hundredths of a second before the succeeding auricular systole. Under normal conditions, the blood flowing toward the heart is sufficient in volume to distend the thoracic veins and the elastic walls of the auricles, which are merely enlargements of the veins. It is this elasticity of the distended walls of the cardiac reservoir, largely assisted in the left heart by the suction of the empty ventricle, and not the contraction of the muscle-fibres in the walls of the auricles, the auricular systole, which fills the ventricles.

When a man lies upon a plank suspended from long wires, each movement of blood feetward into the ventricles or headward into the arteries causes an equal mass-movement of his body and the plank in the opposite direction. By recording these movements (magnified by levers fifty to one hundred times) the quantitative volume-curve of the human heart is obtained. It is similar to those above described.

THE EVENTS WITHIN THE HEART.

By YANDELL HENDERSON.

Since the volume and pressure-curves of my heart-model are (as shown by the tracings presented with the previous paper) essentially similar to those obtained from animals and from man, it may fairly be assumed that the events within this model afford a true picture of the corresponding events within the living heart. Through the courtesy of Professor C. H. Judd, it was made possible by means of a kinetoscope[1] to record the working of the heart-model photographically. Twelve successive pictures, in a total period of six-tenths of a second, show all the events of a cardiac cycle, and also the movements of the stylus of a Hürthle manometer recording intraventricular pressure on a drum. These photographs show, therefore, the pressure-changes, the instant of opening and closing of the valves (especially noteworthy being the closure of the mitral in mid-diastole), and the periods of the filling and discharge of the ventricle.

[1] The kinetoscope was lent to the Yale Psychological Laboratory by the Edison Manufacturing Company.

Further investigations on the model (by the ordinary graphic method) show that the diastolic wave in the intraventricular pressure-curve is due to the inertia of the column of blood which, after pouring through the auricular orifice during the earlier portion of diastole, is brought to a standstill by the full ventricle resisting further distention. The narrower the auricular orifice, and the longer the tube or funnel formed by the flaps of the mitral valve when open, the greater is the pressure which this column of blood exerts in coming to a standstill. This increased pressure intensifies the diastolic wave. It also causes a more sudden and forcible closure (diastolic in time) of the mitral or tricuspid valve. Thus are produced the diastolic sounds incident to mitral stenosis; thus also arise the "weak systoles," alternating with the strong, often noted in mitral stenosis, — the weak and abortive "systole" being truly no systole, but merely an exaggerated diastolic wave.

AN INSTANCE OF COMPLETE "HEART-BLOCK" IN MAN.

By JOSEPH ERLANGER.

Analysis of tracings of the cardiac-impulse, jugular pulse, and brachial pulse obtained from a case of Adams-Stokes disease demonstrates that the primary circulatory disturbances are consequent to a diminution in conductivity at the auriculo-ventricular junction. At most times this block is complete, the ventricular rhythm being totally independent of the rhythm of the venous end of the heart. In one determination the rate of ventricular beats was 27.6 per minute, of auricular beats 98 per minute. It seems probable that the main ventricular systole is followed, after an interval of 0.27 of a second, by an extra-systole, although the evidence for this is not perfectly clear.

The absence of ventricular beats in some parts of the tracings permits of the dissociation of the activities of the auricles from those of the ventricles. In each auricular cycle there appear in the jugular vein one negative and two positive waves. The higher positive wave is undoubtedly produced by the contraction of the right auricle. The deep negative wave that follows it probably results from active dilatation of the auricle. The small but distinct positive wave that precedes the wave produced by auricular systole may owe its origin

to contraction of the great veins. This wave is most distinct when the venous pressure is high.

Each contraction of the auricles sends into the peripheral arteries a small positive wave followed by a distinct negative wave.

The results obtained in an experiment testing the effect of posture upon the rates of the auricles and ventricles indicate that extrinsic nervous influences are brought to bear on the venous end of the heart almost exclusively. For when the patient's posture was changed from recumbent to erect the ventricular rate was increased, but 0.6 of a beat per minute, whereas the auricular rate was increased 15.5 beats per minute.

One tracing obtained after the patient had been in the recumbent posture for some time shows a condition of partial "block" in which the rhythm of the auricle is to the rhythm of the ventricle as 3 is to 1. This relation was maintained when the patient stood, although the heart-rate was increased. While the subject was holding his breath, the condition of partial block gave way to one of complete block. It is possible that this change in conductivity was produced by a venous condition of the blood.

A METHOD OF MEASURING AND RECORDING THE MAXIMUM AS WELL AS THE MINIMUM BLOOD-PRESSURE IN MAN.

By GAYLORD P. CLARK.

To determine the minimum blood-pressure, a wide cuff is applied to the arm with suitable arrangements for increasing the atmospheric pressure within the cuff, which is connected with a straight-tube mercury manometer and a recording mechanism similar to the one reported by Erlanger.[1]

To determine the disappearance and the reappearance of the pulse below the arm-band, and therefore the maximum blood-pressure, a wide cuff is also applied to the forearm, and the entire apparatus employed in connection with the arm, except the manometer, is duplicated for the forearm.

The pressure is applied to each cuff independently, in the following manner: a bottle that can be elevated at will, filled with water, is

[1] ERLANGER: This journal, 1901, vi, p. xxii.

connected through an outlet in the bottom by means of a large rubber tube with the bottom of another bottle stationary upon the table. The air-space in the upper part of the latter bottle is connected with the interior of the cuff. In order to secure uniform increase of pressure in the arm-cuff, the pressure-bottle connected with that cuff is raised by means of a small windlass.

To determine the minimum and maximum pressure of the blood, the pressure-bottle connected with the forearm-cuff is quickly elevated to a height approximating the average minimum blood-pressure (75 mm. Hg), thus producing a good excursion of the lever connected with its recording apparatus, then by means of the windlass the other pressure-bottle is elevated to a point above that at which the pulsations of the forearm-cuff cease, after which it is lowered.

The amount of the pressure is recorded by pinching a rubber tube connected with a tambour as the column of mercury passes each 5 mm. mark on the scale, both in rising and in falling.

The disappearance and reappearance of the pulse in the upper tracing indicates the point of maximum blood-pressure, and the greatest amplitude of the excursions in the lower tracing indicates the point of minimum blood-pressure.

SOME OF THE PHYSICAL PHENOMENA OF MUSCLE-FATIGUE.

By FREDERIC S. LEE.

The investigation of the subject has been continued by the employment of a method in which the isotonic curves of all the contractions of an excised, non-curarized muscle, stimulated at regular intervals, are superimposed upon a recording surface. The differences which were previously [1] pointed out in the mode of fatigue of the muscles of the frog, the turtle, and a mammal, have been confirmed. Lohmann's work, in which the frog's gastrocnemius, on being heated to a mammalian temperature, shows a course of fatigue similar to that of mammalian muscle, has been repeated and found in general correct. But the coracoradialis profundus of the turtle, similarly heated, continues to give its characteristic curve of fatigue. Hence Lohmann's conclusion that there is no physiological difference, in the matter of fatigue, between cold-blooded and warm-blooded muscle, does not seem justified.

[1] Lee: This journal, 1898, ii, p. xi.

Kaiser's method for determining the point on the isotonic curve where the contractile stress terminates, has been employed for the frog's gastrocnemius, and it has been found that as the height of the curve diminishes in the course of fatigue, the contractile stress terminates at progressively lower and lower points. The lowering of the latter does not, however, seem to keep pace with the lowering of the summit of the curve. Hence the two points seem to approach one another.

GELATINE AS A SUBSTITUTE FOR PROTEID IN THE FOOD.

By J. R. MURLIN (read by invitation.)

Dogs were placed in nitrogen equilibrium, at the starvation level, with diets containing proteid, fat, and carbohydrate. Different fractions of the proteid-nitrogen in the food were then replaced by gelatine-nitrogen, and the effects on the equilibrium, as expressed in the percentage of the body-proteid spared, were determined. The calorific requirements of each dog, estimated from Rubner's tables, were supplied in full in all diets. The experiments lasted from two weeks to two months for each dog, the same diet being ingested in periods of from three to five days, separated sometimes by fasting periods of two or three days.

Results agree in showing that it is a matter of indifference how much of the proteid-nitrogen is replaced by gelatine-nitrogen, up to one-half of the starvation requirement. With two feedings per day, the percentage of body-proteid spared does not decrease, even if two-thirds of the proteid-nitrogen are replaced by the gelatine-nitrogen; and perfect balance may be maintained with this fraction, provided the carbohydrates in the diet amount to one-half to two-thirds of the calorific requirements.

In an experiment on a man of 70 kg., a fasting period of three days was followed by a period of the same length during which nitrogenous equilibrium was maintained on a diet containing the quantity of nitrogen eliminated during starvation. The proteid was supplied by beefsteak, oatmeal, and eggs, which, with cream and cane-sugar, furnished a total of 3,000 calories. Two-thirds of the total proteid-nitrogen were replaced by gelatine-nitrogen for two days, with the result that on the second day there was a gain of 0.06 gm. N. The

energy available in this last period was raised by addition of carbohydrate to 3,400 calories. Moderate muscular exercise was taken throughout the experiment.

The conclusion is that a mixed diet, more than covering the heat-requirement of the organism, and containing two-thirds of the starvation minimum of nitrogen in gelatine, and one-third in proteid, will maintain nitrogenous equilibrium for a few days at least. In other words, the proteid requirement under the combined sparing action of gelatine, fat, and carbohydrate falls to one-third the starvation-requirement.

THE EFFECTS OF ISOTONIC SOLUTIONS ON THE KIDNEY.

By TORALD SOLLMANN.

Perfusion of excised kidneys with various solutions of the same freezing point, as 1 per cent sodium chloride, produces the following effects:

Non-Electrolytes. — Cane-sugar causes very slight changes, due to its viscidity and specific gravity. Glucose produces very little effect. Alcohol and urea cause marked diminution of the vein and ureter flow, and of the volume of the kidney. The effect is that of hypo-isotonic solutions; these substances penetrate the cells.

Cations. — Potassium and ammonium produce very little change. Magnesium increases the vein and ureter flow. Barium, calcium, and hydrogen diminish the vein and ureter flow, and the volume.

Anions. — The sulphate, and especially the citrate, increase the vein and ureter flow and the volume. The hydrate, carbonate, and bicarbonate diminish them.

The effects of any of these solutions can be removed by subsequent perfusion with sodium chloride. Mixtures of ions, as far as investigated, produce a combination of the effects. Locke's solution (omitting the dextrose) causes a slight increase of ureter-flow, without change of the vein-flow or areometer.

As far as tried, the effects of any of these solutions can be produced several days after the excision of the kidneys. They are probably osmotic actions, solutions of the same freezing point being anisotonic to the kidney-cells, on account of differences in permeability.

THE EFFECT OF BLOOD ON THE BLOOD-VESSELS OF THE KIDNEY.

By TORALD SOLLMANN.

The perfusion of excised kidneys with viscid solutions (egg-albumen or acacia) results in a marked decrease of the vein and ureter flow, and of the volume of the kidney. The same result occurs on perfusing kidneys with diluted defibrinated blood, several days after excision: in these, the vein-flow is almost stopped. A very different result is seen when recently excised kidneys are perfused: in these, blood causes generally a considerable increase of vein-flow; at the same time, the ureter-flow and renal volume are diminished. The vein-flow may be two or three times greater than with saline solution. This indicates a very pronounced active dilation of the blood-vessels, involving probably the afferent, and especially the efferent arteries. This dilator effect is also produced by blood saturated with carbon monoxide, by blood laked at 63 C., and by serum. It is not produced by Locke's solution, nor by blood coagulated in the steam-bath. It is therefore not produced by the corpuscles, by oxyhæmoglobin, or by the serum salts; but by some substance (proteid?) which is either destroyed or precipitated on heating to coagulation (not at 63° C.). The dilator action is somewhat different, quantitatively, in different samples of blood. The response of different kidneys is also variable; so that in some cases the viscidity effect predominates. The dilator reaction diminishes slowly with the time elapsing after excision, disappearing in eighteen to forty-eight hours, at about the same time as the power to reduce hæmoglobin.

The dilator effect varies with dilution of the blood; but for slight dilution the change is less than that of the viscidity; so that a moderately diluted blood gives a greater vein-flow than an undiluted blood; while a greatly diluted blood may give a lesser vein-flow.

Hydrocyanic acid has also a very marked dilator effect on the renal vessels; but this is confined mainly to the afferent vessels.

Adrenalin may also cause a dilation under certain conditions, whilst its ordinary effect is a pronounced constriction.

FURTHER STUDIES ON RICIN.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

In continuation of the experiments reported at the last meeting,[1] the castor-bean proteids have been separated further by fractional precipitation with neutral salts into portions of relatively great, and relatively slight toxicity, and the ricin prepared in still purer form. The toxic preparations alone possess the power of sedimenting the colored corpuscles of mammalian blood; this property is not lost until the proteid solutions are heated to the temperature of coagulation. The pure ricin preparations suffer no deterioration in their physiological activities after being kept for many months.

Even after administration of quantities many times larger than the fatal dose, a "latent period" of at least fifteen hours intervenes before the toxic symptoms become manifest, as has been noted by other observers. The increase in the dosage is important mainly in determining the rapidity with which the subsequent pathological conditions arise. This fact throws some light on the probable nature of the toxicological action produced by ricin. The toxicity of the pure ricin is enormously diminished when it is administered through the alimentary canal, instead of subcutaneously.

ON THE RATE OF ABSORPTION FROM INTRA-MUSCULAR TISSUE.

By S. J. MELTZER and JOHN AUER.

We came across the observation that absorption from the muscles is incomparably more rapid and efficient than from the subcutaneous tissue, and we tested the fact with several substances. With suprarenal extract we tested it in three ways: (1) By the effect upon blood-pressure: a dose of 0.6 c.c. or less of adrenalin per kilo (rabbit) exerts in subcutaneous injection no effect, and the unstable effects of larger doses consist in a rise of pressure varying between 10 mm. and 20 mm. mercury, which sets in late and develops slowly; while an intramuscular injection of 0.5 c.c. or 0.4 c.c., or even less, per kilo

[1] Osborne, T. B., and L. B. Mendel: This journal, 1904, x, p. xxxvi.

causes invariably a considerable rise of pressure which sets in after a very short latent period, and reaches its maximum in a few minutes. The obtained curve looks very similar to that of an intravenous injection. The rise can be as high as 50 mm. or 60 mm. of mercury and even higher, and the course is frequently interrupted by "vagus-pulses." (2) It was further tested by the effect upon the pupil on the side where the superior cervical ganglion had previously been removed: a dose of 0.5 c.c. or 0.4 c.c. of adrenalin per kilo, given intramuscularly, will cause the dilatation of the pupil in less than a minute, while such a dose, in subcutaneous application, has rarely any effect, and the effect of a larger dose sets in only after ten or fifteen minutes. (3) It was finally tested by the prostrating effect: a dose of 0.5 c.c. per kilo will prostrate a rabbit in a minute or two after intramuscular injection, while in subcutaneous application the effect will not come before twenty or thirty minutes, and then only after much larger doses.

We tested the fact further by means of curare: a dose can be found which will have no apparent effect in subcutaneous injection, but will cause, in a few minutes, paralysis of the voluntary muscles after an intramuscular application.

We have established the striking difference between the effects of the two modes of application also for morphine and fluorescein.

A STUDY OF THE COLORING MATTERS IN THE PURPLE PITCHER PLANT (*SARRACENIA PURPUREA*).

By GUSTAVE M. MEYER and WILLIAM J. GIES.

Two years ago, while studying the digestive powers of *Sarracenia purpurea*, Gies detected a sensitive pigment in aqueous extracts of the pitchers, to which the name *alkaverdin* was given (Journal of the New York Botanical Garden, 1903, iv, p. 37). A few preliminary experiments showed that alkaverdin bore superficial resemblance to the characteristic pigments in red cabbage, elderberry, dahlia, etc. The earlier experiments have lately been extended, with the following general results:

Alcohol extracts three coloring matters from the macerated purple pitchers, — chlorophyl (green), alkaverdin (purplish red), and a brownish-black material. Water extracts only the last two coloring

matters. The brownish-black material may be separated from the alkaverdin in an aqueous extract by evaporating the latter *in vacuo* nearly to dryness. On pouring the residue into absolute alcohol, the brownish-black substance is precipitated. The alcoholic solution contains all of the alkaverdin, and, when evaporated *in vacuo* nearly to dryness, yields a residue resembling dark molasses, both in consistency and color. This syrup is insoluble in ether and chloroform, but is soluble in water and alcohol. The color of the brownish-black material is unaffected by acid or alkali. The substance fails to give a reaction with ferric chloride, and is without reducing power.

The syrup containing the alkaverdin has a bitter taste and sugary odor. The characteristic odor of the macerated pitchers is also present. Halogens, nitrogen, and sulphur are absent. The syrup contains a large proportion of fermentable and dextrorotary carbohydrate, which has a strong reducing action on Fehling's solution, even in the cold, and yields phenyl dextrosazone-like crystals. Sugar can be removed by fermentation without apparent detriment to the pigment. Aqueous solutions of the syrup are reddish in color. When very dilute, the solutions are colorless. Such colorless solutions become *green* when treated with a drop of $\frac{n}{5}$ alkali ("alkaverdin") and are rendered colorless by a drop of $\frac{n}{5}$ acid. A drop or two of acid in excess causes the production of a *pink* color quite unlike the red of the syrup itself. On standing, the pink color is somewhat intensified. In less dilute solutions these tinctorial changes are very striking. Delicate test papers have been made with alkaverdin.

Although no crystalline pigment has yet been separated from the syrup, it is certain that alkaverdin has no effect on the spectrum. On warming its aqueous solutions for some time, on the water bath, alkaverdin is transformed into a brownish product which no longer yields a pink color with acid, but which is still colored green by alkali. After hydration with dilute sulphuric acid (2 per cent), alkaverdin entirely loses its tinctorial properties.

Whether alkaverdin is closely related to chlorophyl or not, has not yet been determined.

A FURTHER STUDY OF PROTAGON.

By E. R. POSNER and WILLIAM J. GIES.

Recent observations by Cramer and by Thierfelder indicate that protagon, as made by Liebreich's or Gamgee's method, contains an admixture of cerebron ("pseudocerebrin"). The latter cannot be entirely removed from the mixture by the usual cooling process. In all probability every product heretofore called protagon has been a mixture of substances, as Lesem and Gies and others have suggested, and it is doubtful whether the name protagon signifies anything definite.

This view is confirmed by our recent experiments. Cramer prepared protagon by a new method, which involved preliminary "coagulation" of the protagon by heating the brain-tissue on the water bath (at 100° C.) with sodium sulphate solution. Protagon was then prepared by Gamgee's method from the tissue which had been treated in this way. In our own experiments, thus far, protagon has been prepared by the Gamgee method and purified by recrystallization from 85 per cent alcohol. The purified products were then heated with sodium sulphate solution, as in the Cramer process. The protagons prepared by the latter method dissolved readily in 85 per cent alcohol at 75° C., and were subjected to the fractionation process (at 45° C.) of Lesem and Gies, with results similar in detail to those published by them. Thus, the phosphorus-content of each successive fraction of the protagon decreased, while the phosphorus-content of the substance in each successive protagon filtrate (at 0° C.) rose far above that of the corresponding protagon, or of any protagon-fraction. The phosphorus-content of the insoluble matter was much lower than that of the original protagon.

Protagons containing as much as 1.7 per cent of phosphorus may be obtained by changing somewhat the method of preparation. Additional facts in this connection will be given at the conclusion of the experiment now in progress.

CERTAIN ASPECTS OF EXPERIMENTAL DIABETES.

FRANK P. UNDERHILL.

With the purpose of extending our knowledge of carbohydrate metabolism, particularly under abnormal conditions, an explanation has been sought for the hyperglycæmia and accompanying glycosuria which can be called forth by experimental methods, — painting of drugs upon pancreas, etc. When piperidin is painted upon the pancreas of dogs, hyperglycæmia is a constant symptom, and it bears a striking similarity to that provoked by adrenalin. The application of the substances directly to the pancreatic cells is not essential, since similar results may be obtained by painting the spleen, by intraperitoneal injection, or by direct introduction into the blood. It seems likely, therefore, that the influence of piperidin, etc., is exerted through the intervention of the circulation rather than directly upon the gland-cells to which the substances have been applied. The experimental diabetes is not due to an irritant action upon, or an "insult" to, the pancreas. The hyperglycæmia may be induced independently of the pancreas. The experiments indicate that the action of piperidin, etc., is not specific; nor is the pharmacological action of the drugs necessarily related to their chemical structure. The tentative explanation offered for the hyperglycæmia and glycosuria provoked by such substances as piperidin, potassium cyanide, ether, chloroform, morphine, carbon monoxide, strychnine, pyrogallol, pyrrol, pyridin, coniin, nicotin, curare, etc., and possibly adrenalin, is not that they have any specific action (such as deprivation of oxygen) upon any particular gland like the pancreas, but that these substances have more or less effect upon the respiratory centre in producing dyspnœa. Dyspnœa calls forth a marked hyperglycæmia and glycosuria without the intervention of any drugs. The typical rise in the sugar-content of the blood produced by piperidin fails to appear when oxygen is administered. It seems probable, therefore, that this experimental diabetes is due to diminished oxidation of carbohydrate material, with the consequent accumulation of the latter in the blood, and its elimination by the kidneys.

Some Observations on the Catalytic Decomposition of Hydrogen Peroxide by Organ Extracts. By A. S. Loevenhart.

On the Theory of Geotropism in Paramœcium. By E. P. Lyon.

The Nitrogen of Urine; its Distribution among the Four Important Constituents — Urea, Ammonia, Uric Acid, Kreatinin. By O. Folin, (By invitation).

On Psychic Secretion. By W. Koch.

The Pharmacology of Ethyl Salicylate. By E. M. Houghton.

Demonstration of a New Water Manometer. By F. S. Lee (for H. Emerson).

An Adjustable Tracheal Cannula for Artificial Respiration. By G. P. Clark.

An Exhibition of Physiological Apparatus. By W. T. Porter.

Demonstration of Apparatus. By E. P. Lyon.

Demonstration of Apparatus. By A. P. Brubaker.

The following communication was read by title:

A Second Coagulation of the Blood due to a Substance not Identical with Fibrinogen, and Coagulable by Saturation with Neutral Oxalate. By E. T. Reichert.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 159.

THE REVERSAL OF CILIARY MOVEMENT IN METAZOANS.

BY G. H. PARKER.

I. INTRODUCTION.

THE reversal of ciliary movement, though one of the commonest phenomena among protozoans, seems to be of much rarer occurrence among the higher animals. In a recent review of the problems of ciliary movement, Pütter (:03, p. 35) devotes only two paragraphs to ciliary reversal in metazoans, nor does the literature on the subject warrant a more extended treatment.

Apparently Purkinje and Valentin ('35, p. 67), who observed spontaneous ciliary reversal on the accessory gills (Nebenkiemen) of the mussel, were the first to record this condition in metazoans. Their statements were repeated by Valentin ('42, p. 513) in his article on cilia in Wagner's Handwörterbuch der Physiologie, and were confirmed later by Engelmann ('68, p. 476; '79, p. 386; '98, p. 788). According to Miklucho-Maclay ('68, p. 232), some sponges can temporarily reverse the direction of their water-currents, an observation subscribed to by Haeckel ('72, p. 26), and indicating a probable reversal in the action of their flagella. Minot ('77, p. 407) observed that the cilia at the head ends of certain fresh-water planarians strike in various directions, while those on other parts of the body always strike backward, and Iijima ('84, p. 436) noticed that certain bands of cilia in Dendrocœlum, Planaria, and Polycelis strike now in this direction,

now in that. Probably the cilia of many of the smaller water-inhabiting metazoans reverse, as recorded by Kleinenberg ('86, p. 22), for the larvæ of Lopadorhynchus, and by Schulze ('91, p. 13), for Trichoplax. Finally the labial cilia of certain actinians reverse during feeding, as shown by me (Parker, '96, p. 114) in Metridium marginatum, by Vignon (:01, pp. 638, 656), in Sagartia parasitica, and by Torrey (:04, p. 212), in Sagartia davisi. Engelmann ('79, p. 386) stated that he never observed ciliary reversal in any of the vertebrates; but Roux ('95, p. 106) recorded an instance of the reversal of the direction of rotation in a frog embryo that may have been due to this cause.

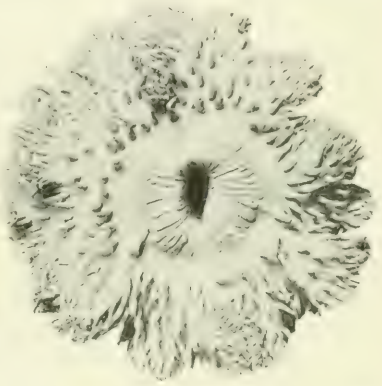
Thus only a few scattered cases of ciliary reversal in metazoans have been put on record, and these only in the briefest way. In the present investigation I have attempted to work out, in more detail than has been done heretofore, the conditions of reversal, and for this purpose I have experimented with the labial cilia of the actinian Metridium marginatum.

II. Observations.

In Metridium marginatum the oral disk consists of three concentric areas which from the periphery inwards are as follows: the tentacular zone (see figure), bearing the numerous tentacles; the intermediate zone, without special organs; and the labial zone, represented by the swollen lips and siphonoglyphs, which together surround the mouth. The tentacles, lips, and siphonoglyphs are well ciliated; otherwise the oral disk is without cilia or at most sparsely provided with them.

In an expanded resting Metridium the mouth is usually somewhat open, the lips are protruded, and the tentacles are recumbent and point away from the centre of the disk. Under such circumstances the effective stroke of the cilia can be demonstrated, by means of carmine particles, to be inward on the siphonoglyphs, outward on the lips, and outward on the tentacles, *i. e.*, toward their tips. Thus currents pass into the animal at the siphonoglyphs, and out of it over the lips and tentacles. In all the trials that I have made on the directions of currents in quiescent animals, those just stated have invariably been met with, and I, therefore, have no grounds for supposing that any of these ciliated parts of Metridium show spontaneous reversals such as have been claimed to occur on the gills of mussels.

So far as my experience extends, the application of various stimuli to the tentacles has never resulted in a reversal of the effective stroke of their cilia, and the same is true of the siphonoglyphs. On the lips, however, as I have elsewhere shown (Parker, '96, p. 113), ciliary reversal may be accomplished by the application of appropriate stimuli. The normal outward stroke of the labial cilia is not reversed by carmine, India ink, sand, or pellets of filter-paper moistened with sea-water; or with solutions of sugar, quinine, or picric acid in sea-water; but it is reversed when pieces of crab meat are applied to the lips. That this reversal is dependent upon the dissolved substances in the meat is seen from the fact that a pellet of filter-paper, which when moistened with sea-water is carried outward, is swept inward when soaked with meat juice. Moreover, a piece of crab meat that has been so thoroughly washed in sea-water as to have lost almost all its extractives will usually not induce reversal. It therefore appears to be certain that the reversal caused by crab meat is dependent upon certain dissolved substances contained in the meat.



Oral view of an expanded specimen of Metridium marginatum, showing the tentacular zone (outermost), the intermediate zone, and the labial zone (surrounding the mouth). The specimen, which was diglyphic, was prepared by Semper's method and is reproduced natural size.

Another piece of very conclusive evidence in favor of this view can be obtained from microscopic preparations. When a piece of the lip is placed in sea-water under a microscope, the normal direction of the ciliary stroke can be easily observed. If now such a preparation is flooded with sea-water containing meat juice, an immediate reversal can be seen to take place, and on washing the preparation subsequently with pure sea-water, a return to the normal direction occurs. Such changes can be repeated many times without impairing the piece of tissue. Hence there can be no doubt that the dissolved substances in the meat cause ciliary reversal.

The juice obtained from the meat of the common green crab, Carcinus mænas, which was used in these experiments, was in large part blood, and hence it seemed not improbable that the

blood of this animal contained some substance or substances that caused the reversal. Whether these were organic or inorganic could not be foretold; but because of the greater ease with which pure inorganic substances could be obtained, I resolved to test these first.

According to Griffiths ('92, p. 293), the blood of Carcinus contains, besides traces of iron and copper, sodium, potassium, calcium, and magnesium in the form of chlorides, phosphates, and sulphates. As a preliminary test, solutions in sea-water of the more soluble phosphates and sulphates and of the chlorides of all four metals were prepared and tried on the labial cilia under the microscope. The strengths used varied from 10 per cent to 1 per cent.

No clear evidence of reversal was obtained from any of the solutions tested except from that of potassic chloride. The following records from one series of experiments with the solutions of this salt will suffice to make clear its effects and range.

10 per cent potassic chloride in sea-water.

When applied, much mucus was discharged and many nettle capsules were exploded.

15 min. 33 sec. after application, ciliary movement ceased. The tissue was then rinsed in pure sea-water and immersed in it.

15 min. 27 sec. after immersion, there having been no signs of recovery and the cells having been apparently killed, the experiment was discontinued.

5 per cent potassic chloride in sea-water.

When applied, some mucus was discharged and many nettle capsules were exploded.

5 min. 30 sec. after application, the first irregular ciliary movements were observed.

4 min. 30 sec. later, ciliary reversal was well established, though the mucus and exploded nettle capsules impeded the cilia.

5 min. later, the material was transferred to pure sea-water in which ciliary movement continued, but so obscured by mucus that the direction could not be determined.

4 per cent potassic chloride in sea-water.

When applied, some mucus and nettle capsules were discharged and the cilia ceased moving.

1 min. 45 sec. after application, ciliary movement was renewed in irregular form.

3 min. later, ciliary reversal was well established, though the action of the cilia was encumbered by mucus.

10 min. later, pure sea-water was applied and ciliary action ceased.

6 min. 15 sec. later, the first irregular ciliary movements began.

9 min. 15 sec. later, normal ciliary movement was re-established, but not as vigorously as at first.

3 per cent potassic chloride in sea-water.

When applied, nettle capsules were discharged.

1 min. later, irregular ciliary movements were observed.

2 min. 45 sec. after the first irregular movements, complete reversal was established.

10 min. 15 sec. after this, the tissue was placed in pure sea-water. Ciliary action ceased.

3 min. 45 sec. later, irregular local movements began.

9 min. 45 sec. later, normal movement was vigorously re-established.

2 per cent potassic chloride in sea-water.

30 sec. after application, reversed ciliary movement was well established. Some mucus and nettle capsules had been discharged.

12 min. 40 sec. later, the tissue was flooded with pure sea-water.

1 min. 50 sec. later, irregular movements began.

4 min. 20 sec. later, vigorous normal movement was re-established.

1 per cent potassic chloride in sea-water.

15 min. 45 sec. after application, normal movement still continued. Some mucus and nettle capsules had been discharged.

2 min. 15 sec. later, the potassic chloride solution was renewed.

30 min. 30 sec. after the first application, the normal movement was still unchanged and the experiment was discontinued.

From these records it can be seen that potassic chloride is a stimulant for the production of mucus and the discharge of nettle capsules, and that these two results are at times so excessive as to hinder or at least obscure the action of this salt on the cilia. A 1 per cent solution is apparently not strong enough to cause ciliary reversal, and a 10 per cent solution soon kills the ciliated cells. The most successful concentration of those just described, whereby reversal could be effected, was 2 per cent, for by this solution reversal was not only quickly accomplished, but the production of mucus, etc., was minimized. Subsequent experiments demonstrated that 2.5 per cent was still better than 2 per cent, and this strength was therefore used in much of the later work.

When care was taken to prevent mucus from accumulating on the pieces of tissue, the reversal by means of a 2.5 per cent potassic chloride solution could be brought about as quickly and with as much certainty as with crab-meat juice. Moreover, the artificial fluid would, without great damage to the tissue, induce repeated reversal

of the ciliary stroke as the natural fluid did. Thus, in an experiment lasting somewhat over four hours, a single preparation was reversed five times by 2.5 per cent potassic chloride and brought back to its normal state by pure sea-water without apparently suffering any serious injury, for at the end of this experiment the preparation was still vigorous. The five intervals between the moment of applying the salt solution and the occurrence of reversal, and those between the application of pure sea-water and the return to the normal stroke were, as the following table shows, all brief, and in this respect resembled the changes as produced by the natural fluids.

TABLE.

Times in Minutes and Seconds for the Reversal (in 2.5 per cent KCl) and the Return to Normal (in Pure Sea-water) of the Ciliary Stroke.

No. of Experiment.	1		2		3		4		5	
	min.	sec.	min.	sec.	min.	sec.	min.	sec.	min.	sec.
In 2.5 per cent potassic chloride.	0	30	2	15	2	0	1	5	2	30
In pure sea-water.	10	25	9	0	13	10	10	20	12	10

Although it must be admitted that potassic chloride is in some way the cause of the reversal of the ciliary stroke, a further analysis of this question requires a consideration of somewhat more artificial conditions. In accordance with the dissociation-hypothesis an aqueous solution of potassic chloride consists of potassium ions, chlorine ions, and a certain amount of undissociated potassic chloride. It is possible that any one of these three bodies, or some combination of them, may cause the reversal of the cilia. To ascertain upon which of these the reversal depends it was found necessary to devise some chemically simple solution in which the cilia would continue to live, and which might therefore be used as a substitute for the highly complex mixture, sea-water. After some few trials, it was found that the cilia would survive several hours in a $\frac{5}{8}$ *m* solution of sodic chloride. When to this was added enough potassic chloride to make a $\frac{1}{3}$ *m* solution (= *circa* 2.5 per cent) of that salt, a relatively simple chemical mixture was obtained, by means of which ciliary reversal could be invariably accomplished, though at a slower rate than by potassic chloride in sea-water. Thus the ciliary stroke of a fragment of a lip was reversed by an immersion of somewhat less than fifteen minutes in a solution containing $\frac{5}{8}$ *m* NaCl and $\frac{1}{3}$ *m* KCl, though a second frag-

ment from the same lip used in a check experiment retained its normal direction of strokes for two and a half hours in a $\frac{5}{8}$ *m* NaCl solution. In a second experiment the reversal was accomplished in eight minutes, and the reversed stroke was maintained for over thirty-five minutes, till the preparation was clogged with mucus.

By using a solution of $\frac{5}{8}$ *m* NaCl in place of sea-water, and by adding other salts to this solution, it was now possible to proceed to experiments for the elucidation of certain other questions. Since the cilia will continue their normal stroke for some time in a solution of $\frac{5}{8}$ *m* NaCl to which has been added $\frac{1}{3}$ *m* NaCl, but reverse it in one to which has been added $\frac{1}{3}$ *m* KCl, it follows that this reversal cannot be due to the osmotic differences of these two solutions, for they are practically isotonic, the pure sodic chloride solution having an osmotic pressure equal to 36.0 atmospheres, and the mixture having one equal to 36.9 atmospheres. It is also improbable that the anions are concerned with the ciliary reversal, for the degree of dissociation of the two solutions, 67.9 per cent in the pure sodic chloride, and 74.0 per cent in the mixture, shows that the state of the chlorine is nearly the same in each. Moreover, no reversals were obtained with $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* LiCl, or with $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* NH_4Cl, in both of which the chlorine content is not very unlike that of the reversing fluid. These fluids had very different actions on the cilia; that containing lithium soon brought them to a standstill, while the one with ammonium in it had little or no effect on their movement.

If the osmotic pressure and the chlorine ions are not the cause of ciliary reversal, this must be sought for in the potassium ions or the molecules of potassic chloride. Which of these was the probable cause was ascertained by subjecting the cilia to the action of solutions containing other potassium salts than the chloride and noting whether reversal occurred or not. A reversal similar to that observed in the potassic chloride solution was obtained in six minutes from a solution composed of $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* KNO_3. Since no reversal was produced by a solution containing $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* $NaNO_3$, it is clear that the reversal is caused by the potassium ions. Hence in this instance the cation is the stimulating component, though Mathews (:04, p. 456) has recently endeavored to show that these ions are depressing in their action and anions are stimulating.

If the potassium ions must be admitted to be the means of reversing the effective stroke of the cilia in the experiments just described,

it might be assumed that, since they are also in the blood of the crab, they are the active agents in the reversal produced by that material. This conclusion, however, is probably not entirely true, for the action of the potassic chloride solution is at least in two respects different from that of the meat juice. The potassic chloride solutions always induce a considerable secretion of mucus and a discharge of many nettle capsules, reactions not especially noticeable with the meat juice. Further, a 1 per cent solution of potassic chloride will not reverse the ciliary stroke though a dilute solution of meat juice, which must contain very much less than 1 per cent of potassic chloride, is very effective in this respect. Because of these differences it seems improbable that the reversal by meat juice is due simply to the contained potassium. Possibly potassium, in combination with organic materials, is the really effective agent in bringing about the reversal, but on this point I have no conclusive evidence to offer.

Torrey (:04, p. 212) observed that in Sagartia davisi such chemically inert substances as paraffine, glass, paper, etc., were swallowed, and he (:04, p. 210) believed that these substances cause a ciliary reversal by purely mechanical means. In my own experiments on Metridium, though I have looked with care, I have never observed a ciliary reversal due to such stimulation. Perfectly insoluble substances like pellets of filter-paper, etc., are always swept outward on the lips. In some of my earlier experiments I noticed occasionally that a filter-paper pellet supposed to be moistened with sea-water only, would take a somewhat ambiguous course, moving for a short time inward and then outward. By further experiments I convinced myself that these apparent exceptions were due to organic contamination from my fingers, for when care was taken that the pellets should consist of nothing but clean filter-paper wet with sea-water no such reversals occurred. Torrey (:04, p. 210) has suggested that possibly the inward movement of pieces of India rubber observed by me on the lips of Metridium might be evidence of the reversal of the ciliary stroke in that sea-anemone by mechanical means. I have, therefore, repeated my experiments with small fragments of white India rubber such as is used for laboratory tubing, and I find, as I previously stated (Parker, '96, p. 115), that these pieces are occasionally swallowed, and are almost always carried inward some distance before they are finally discharged. Filter-paper pellets, however, that have been soaked in a filtrate from a mixture of sea-water and many small pieces

of rubber, call forth the same reactions, and I therefore conclude that this response is really dependent on dissolved products from the rubber. Thus while the labial cilia of Sagartia davisi may be reversed mechanically, I know of no conclusive evidence in favor of this means in Metridium.

It has long been known to students of ciliary activity that the direction of the effective stroke of a cilium, *i. e.*, the direction in which the adjacent fluid is moved, is independent of the direction of what may be called the propagation wave, *i. e.*, that disturbance which determines the *sequence* of stroke of the individual cilia in a general field. Thus the direction of the effective stroke of the cilia on the frog's palate is inward, as is also the direction of their propagation wave. On the other hand, the direction of the effective stroke of the swimming plates of ctenophores is toward the aboral pole, that of their propagation wave toward the oral pole. Thus the two phenomena are independent so far as direction is concerned, since, in the first instance, the directions agree, in the second they are opposed.

In the normal beat of the labial cilia of Metridium, the effective stroke and the propagation wave agree in direction in that both are toward the exterior. From what has been said, it is conceivable that when the effective stroke is reversed, the propagation wave may or may not be reversed. To ascertain what happened under these circumstances, I made the following experiment. A piece of Metridium lip was placed in sea-water under the microscope, and the normal direction of the stroke was noted. A small piece of crab meat was then attached to the end of a glass rod and brought in contact with the ciliated lip. In a very short time the cilia immediately under the meat could be seen to have reversed their effective stroke, though those in front of and behind the meat were beating in the normal direction. In the region of the reversed cilia not only had the direction of the effective stroke changed, but the propagation wave had also reversed. The lines of separation between the reversed area and the normal areas were rather sharp and located just at the edges of the applied meat, showing that the reversal of both the effective stroke and the propagation wave was a strictly local phenomenon and dependent upon local stimulation. Since the reversal includes not only the direction of the effective stroke, but also that of the propagation wave, it is probable that the potassium ions or other reversing materials stimulate not only the cilia, but also the deeper lying cyto-

plasm of the ciliated cells through which the propagation wave is transmitted.

The strictly local character of the response, as indicated in the preceding experiment, agrees with my previous observations (Parker, '96, p. 114) on the whole lip of Metridium, and leads to a conclusion, expressed in my former paper, that these experiments yield no evidence in favor of the view that the labial cilia are under nervous control. I see no reason for assuming, as Vignon (:01, p. 659) has done, that the reversal is dependent upon nervous operations in the nature of central reflexes.

Perhaps one of the most noteworthy results of the present investigation is the fact that though the ciliary stroke on the lips of Metridium is quickly reversed by weak crab-meat juice, that of the siphonoglyphs and tentacles is unaltered in direction by this material. These conditions point to a high degree of differentiation in the ciliated surfaces of Metridium and emphasize the general truth that ciliated surfaces are closely adapted to their special environments. That such surfaces have much individuality is clearly seen from the fact that the labial cilia of Metridium will live several hours in a pure $\frac{5}{8}$ *m* sodic chloride solution and reverse in one to which $\frac{1}{3}$ *m* potassic chloride has been added, though in both these solutions, according to Lillie (:01, pp. 62, 65; :02, p. 41), the cilia of Arenicola larvæ are almost immediately destroyed, and those of Polygordius larvæ are soon broken down. On the other hand, lithium chloride seems to act uniformly on the cilia of all these animals, and quickly checks their action; whereas ammonium chloride has little or no serious effect. (Compare the statements made by Lillie, :04, p. 422, and on page 7 of this paper.) These observations show the necessity of wide experimental acquaintance with the reactions of cilia before general conclusions of much value can be arrived at.

Whether ciliary reversal is a phenomenon of wide or restricted occurrence among metazoans is a question still to be answered. The part that it plays in the feeding habits of actinians suggests that it may be a factor of like import in the feeding and respiratory activities of many of the lower animals, such as the lamellibranchs, etc. It is also possible that reversal may occur in the ciliated tubes and cavities of the higher animals. Thus the transportation of the spermatic fluid in those vertebrates with internal fertilization would be facilitated, if among the glandular secretions of the males material was found that would induce temporary ciliary reversal in the oviducal tracts of

the females. But so far as I am aware, experimental evidence in these directions is almost entirely lacking.

III. Theoretic Considerations.

Although the extreme minuteness of cilia has made a direct investigation of their finer anatomy practically impossible, the study of their action has led to certain fairly well founded conclusions as to their probable structure. The old opinion that cilia are not motile, but are composed of an elastic substance, and are moved by a mechanism at their bases, though lately revived in a measure by Benda (:01), has not appealed as a rule to recent workers. The striking correspondence in chemical reactions, etc., between cilia and living protoplasm, the close relation between protoplasmic pseudopodia, flagella, and cilia, and the continued activity of isolated cilia, have all supported the belief that the cilia themselves are actively contractile. Nor is there anything in recent work that especially favors Schäfer's ('91, :04) belief that ciliary movement is produced by the injection of fluid into an elastic sheath and its subsequent withdrawal. In fact, it is now generally conceded that these organoids contain within themselves their own contractile material.

Such contractile material would act most efficiently if combined with some skeleton-like structure for mechanical support. As Pütter (:03, p. 32) has pointed out, the most effective place for the contractile material is the periphery of the cilium, the axis of which would therefore be the most advantageous position for the support. It is probable, as Pütter (:03, p. 37) has stated, that the axial fibrils described by Ballowitz ('88, '90) in the tails of spermatozoa, and believed by him to be contractile material, are really the supporting substance, and that the more peripheral surrounding layer is the true contractile portion. That an axial portion, such as Ballowitz has identified in spermatozoa, occurs in the minute cilia of ciliated epithelia has never, to my knowledge, been demonstrated; but the action of these organoids certainly suggests the presence of such a part, in which case its occurrence ought to be characteristically different for the two kinds of cilia, reversible and irreversible.

In ordinary cilia incapable of reversing, the contractile layer ought to be best developed on the face of the cilium corresponding to the direction of the effective stroke. On the face opposite this, only

enough contractile material would be necessary to bring about a return of the cilium to the position at which the effective stroke begins, and even this substance might be done away with provided the supporting structures had enough elasticity for the return of the cilium. The absence of contractile material from the sides of the cilium would restrict its movement to a single plane. Such a cilium has been likened to a vertebrate limb, in that it would possess an internal skeleton and flexor and extensor elements, though it may be that the extensors are replaced by the elasticity of the skeleton. But whether we postulate extensors, or only an elastic skeleton, the ultimate structure of such a cilium must be unsymmetrical in that the contractile material must be gathered especially on one side of the supporting axis.

With reversible cilia, the ultimate structure must be quite different. These cilia must consist of flexor and extensor elements placed on the opposite sides of the supporting axis, and about equally developed, but so specialized that, to take an example from the labial cilia of Metridium, in ordinary sea-water, what we may call the flexor elements are stimulated to excessive action, and in sea-water containing potassium ions the extensor elements are more efficient. Reversible cilia, therefore, should have a more symmetrical form than the irreversible ones, but their contractile materials would be more highly specialized than those of the irreversible cilia.

What the exact nature of this contractile material may be is quite unknown, but I see no reason to suppose that it may not be delicately fibrillar, as suggested by Engelmann ('68, p. 456). Pütter ('03, p. 32) has opposed this hypothesis because of the difficulties met with in applying it to the complex and often variable movements of flagella, and has argued in favor of a more nearly homogeneous material in which the particles may temporarily arrange themselves in lines in various directions. But if Engelmann's hypothesis is not wholly satisfactory, Pütter's seems to me still less so, for it contains no suggestion as to what determines the new linear arrangements of particles, and thus leaves unanswered the real question at issue. In my opinion, it is entirely possible that, while the contractile layer of some flagella may be almost homogeneous, that of the more regularly beating cilia is very probably fibrillar, for certainly the movements of these two kinds of organoids are different enough to warrant a difference of structure. I therefore do not agree with Pütter in discarding the fibrillar hypothesis of ciliary structure because it is

inconsistent with some of the facts of flagellar movement; but on the contrary I believe that this hypothesis is the most consistent thus far advanced, as an explanation of the more usual type of ciliary movement, — a movement which in its regularity differs widely from that of most flagella.

IV. Summary.

1. The labial cilia of Metridium marginatum do not reverse when in contact with carmine, India ink, sand, pellets of filter-paper moistened with sea-water, or with solutions of sugar, quinine, or picric acid in sea-water. They also do not reverse as a rule to crab meat from which most of the extractives have been taken.

2. They reverse to dilute crab-meat juice and slightly to a sea-water extract of rubber. They also reverse to a $2\frac{1}{2}$ per cent solution of potassic chloride in sea-water.

3. They will live several hours in a pure $\frac{5}{8}$ *m* NaCl solution, and reverse in a solution containing $\frac{5}{8}$ *m* NaCl and $\frac{1}{3}$ *m* KCl.

4. Reversal is not due to the osmotic action of the reversing fluid, for they reverse in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* KCl (36.9 atmospheres), and not in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* NaCl (36.0 atmospheres).

5. Reversal is not due to the anions, for it occurs in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* KCl, but not in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* NaCl, $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* LiCl, or $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* NH_4Cl.

6. Reversal is due to potassium ions, for it occurs in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* KNO_3, but not in $\frac{5}{8}$ *m* NaCl + $\frac{1}{3}$ *m* $NaNO_3$.

7. The reversal due to crab-meat juice is probably dependent upon some organic combination containing potassium.

8. There is no evidence of spontaneous reversal or of reversal through mechanical stimuli.

9. In reversal the propagation wave, as well as the effective stroke, change directions.

10. The reversal occurs only where the stimulus is applied, and gives no evidence of involving nervous reflexes.

11. Though crab-meat juice causes the labial cilia to reverse, it does not alter the direction of the ciliary stroke on the tentacles or the siphonoglyphs, thus demonstrating the extreme differentiation of these surfaces to stimuli.

12. Irreversible cilia are probably unsymmetrical, in that they consist of a supporting elastic element, on at least one side of which contractile material is present.

13. Reversible cilia are probably more nearly symmetrical, in that there must be contractile material on opposing sides of a supporting element; but these two portions of contractile material must be regarded as chemically different, in that one becomes predominantly active in pure sea-water, and the other in sea-water containing crab-meat juice or potassium ions.

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EXPERIMENTAL STUDIES ON THE PHYSIOLOGY OF THE MOLLUSCS. — FIRST PAPER.

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IT is probable that no group of the lower animals has attracted so much attention and furnished the means for such numerous investigations along physiological lines as the Mollusca. There is no group of the invertebrates or of the lower vertebrates that lends itself so readily to the experimenter, that is found so widely distributed, and that offers at the same time such a wide degree of variation and specialization. Between the slowly moving, thoroughly protected lamellibranchs and gasteropods, with their rudimentary sense organs and their slow, uncertain response to almost all forms of stimuli, and the cephalopods depending entirely for protection and food upon their highly perfected sense organs and their speed of motion and reaction to stimuli, there exists an enormous range of variation. Indeed the difference between the lower and higher members of this group is scarcely less than that which exists between the lowest and highest vertebrate. No other group has adapted itself so thoroughly to meet the demands of such varied types of environment; and it is for this reason that a comparative study of its various members in relation to their environment is especially interesting.

In the present series of studies on the physiology of the molluscs, Sycotypus canaliculatus has been selected as the subject of investigation for several reasons. In the first place, the large size and common occurrence of this gasteropod along the Atlantic coast greatly facilitates the study; and secondly, it represents a type of mollusc that has hitherto been neglected. Considerable work has been done upon the herbivorous gasteropods such as Helix and Limax, as well as upon the pulmonates and lamellibranchs, while still more is known about the physiology of the cephalopods, the most obvious examples

of carnivorous molluscs;[1] but so far as can be learned no carnivorous gasteropod, of which Sycotypus is one of the most extreme types, has been thus examined.

Sycotypus is found abundantly in Long Island Sound and the adjacent waters, frequenting the oyster beds, where it finds its food. When unable to secure this otherwise, it is believed to be able to bore through the shell of its prey and suck out the contents in the same way in which the common "drill" obtains its food. Associated with it, but occurring in smaller numbers, is the closely allied form Fulgur carica. Our studies have shown no distinct physiological differences between the two; and the descriptions of experiments upon Sycotypus apply equally well to Fulgur. Both are thoroughly protected by their shells and opercula, are extremely slow of movement, and are provided with a simple and comparatively low form of nervous and sensory mechanism. Metabolism of all kinds is slow; digestion, respiration, muscular movement, and reaction are of a low order, corresponding to the limited demands of the environment with which the animal associates itself.

The Physiology of the Alimentary Canal of Sycotypus canaliculatus.

Inasmuch as any description of the physiology of an organ implies familiarity with its anatomy, it has been deemed advisable to introduce a brief discussion of the anatomy of the alimentary canal of Sycotypus in connection with the study of its functions. For the general anatomical arrangement, the diagram will perhaps serve the purpose better than a more exact figure. The names used throughout are those commonly employed by morphologists[2] for the designation of the various organs. They refer evidently to analogies of posi-

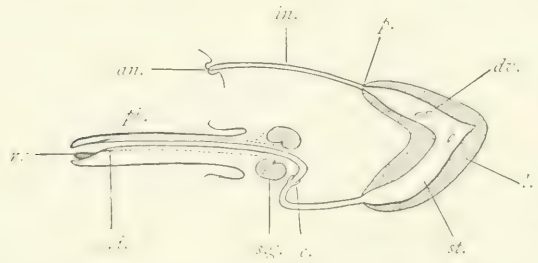


FIGURE 1.— Diagram of the digestive tract of Sycotypus. *pb.*, proboscis; *s. g.*, salivary gland; *c.*, crop; *st.*, stomach; *l.*, liver; *dv.*, hepatic ducts; *p.*, pylorus; *in.*, intestine; *an.*, anus; *s. d.*, salivary duct; *r.*, radula.

[1] The literature on this subject is well reviewed in v. FÜRTH's Vergleichende chemische Physiologie der niederen Tiere.

[2] *Cf.* BUMPUS: Invertebrate Zoölogy.

tion merely, and are no indication, *per se*, of the function of an organ.

The anterior end of the alimentary canal of Sycotypus, as of other allied carnivorous gasteropods, is prolonged to form a movable proboscis which can be withdrawn within the head proper, or extended from two to three inches beyond. Extension is accomplished by the turgescence of the tissues, while flexion and retraction are effected by the flexor and retractor muscles of the proboscis, and by the contraction of the outer circular muscle fibres, by which the blood may largely be removed from the vascular spaces of the organ.

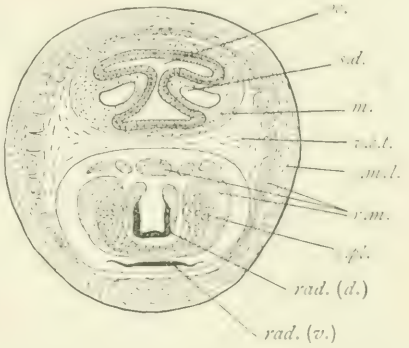


FIGURE 2. — Cross section of the proboscis. *oe.*, œsophagus; *s. d.*, salivary duct; *m.*, muscular coat; *v. c. t.*, vascular connective tissue; *c. m. l.*, circular muscular layer; *r. m.*, retractor muscles; *c. pl.*, cartilage plate; *rad.* (*d.*), radula (dorsal); *rad.* (*v.*), radula (ventral).

The mouth proper is provided with the chitin-like radula, and the openings of the salivary ducts. By means of the former, playing over the end of a cartilaginous plate, and drawn backward and forward by the radulary muscles, Sycotypus is enabled to penetrate the shells of its prey, and to comminute its food.[1] The ducts, opening into the mouth on either side,

[1] It is a matter of physiological interest to note that although hæmoglobin is lacking in the blood of this animal, being replaced by a copper-bearing respiratory proteid, — a hæmocyanin, — the muscles operating the radula are brightly pigmented with hæmoglobin. With the exception of a slight amount in the heart musculature, and in the ganglia and main nerve trunks, no other tissues of the body contain this latter pigment. Undoubtedly the regular contractions of these muscles for long periods of time, as during the process of shell perforation, or the comminution of food, require a more thorough tissue respiration than the sluggish stream of hæmocyanin could furnish. By means of the hæmoglobin, these tissues may be enabled, during the periods of rest, to store up oxygen sufficient for the excess of demand over supply during work. In other words, the presence of hæmoglobin in these muscles strongly suggests an adaptation to the heightened internal respiration attendant upon their unusual contractile changes. This same local pigmentation with hæmoglobin is not confined to this particular species. The "drill," Urosalphinx cinerea, which operates in much the same way, is similarly provided with hæmoglobin-pigmented radulary muscles, while many other molluscs have ganglia and nerve trunks similarly pigmented.

carry the secretion from the salivary glands: the two large, yellow, secreting glands of the head. It has been assumed from the size and position of these glands that they secrete a solution to assist in shell-perforation, — possibly hydrochloric acid or some similar solvent for calcium salts. As will be demonstrated later, this is not the case; the function of the salivary secretion appears to be primarily digestive.

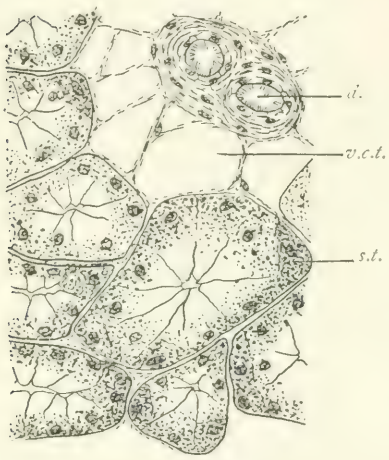


FIGURE 3. — Section of salivary gland. *d.*, duct; *v. c. t.*, vascular connective tissue; *s. t.*, secreting tubules.

From the mouth, the œsophagus extends along the dorsal side of the proboscis (*cf.* Fig. 2), passes through the circum-œsophageal ganglia, bends sharply to follow the general spiral arrangement of the animal, and enters the stomach close to the pericardial sac. Near the ganglia, the œsophagus is invaginated to form the crop, — a small organ of obscure function.

Section of the œsophagus anywhere along its length, shows it to be a tube of simple ciliated epithelium, rather rich in mucous cells, and surrounded by a thick muscular layer consisting chiefly of circular fibres.

Its function is apparently comparable with that of the œsophagus in higher animals, — *i. e.*, the transportation of the insalivated food mixture from the mouth to the stomach. As was demonstrated experimentally, the mucosa apparently contains neither enzymes nor activating kinases.

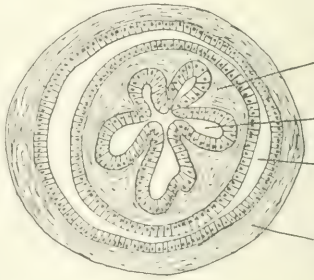


FIGURE 4. — Section of crop. *m. l.*, muscular layer; *lum.*, lumen; *l. c.*, lumen of crop.

The stomach is that portion of the alimentary canal which lies superficially embedded in the liver, or so called hepato-pancreas. It forms a distinct loop, with a shallow cæcum at the angle. Its lumen is considerably greater than that of the œsophagus or of the intes-

tine proper. At the same time a sheath of vascular connective tissue in part replaces the muscular coat so prominently developed in the œsophagus. Peristaltic movements of the stomach are minimal, and are employed chiefly in the evacuation of the undigested residues after digestion is completed.

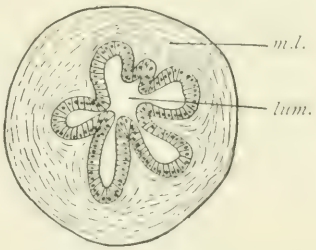


FIGURE 5. — Section of œsophagus. *m. l.*, muscular layer; *lum.*, lumen.

The most characteristic feature of the stomach mucosa is the number and complexity of the rugæ, which nearly fill the lumen of the cardiac portion, where they are most highly developed. Their function is evidently to retard the progress of the digestion mixture through the stomach and to offer a maximum surface for absorption. They are thus quite analogous to the intestinal villi of the vertebrates. The products of proteolysis and, to a much less degree, fats and carbohydrates are presumably absorbed by them.

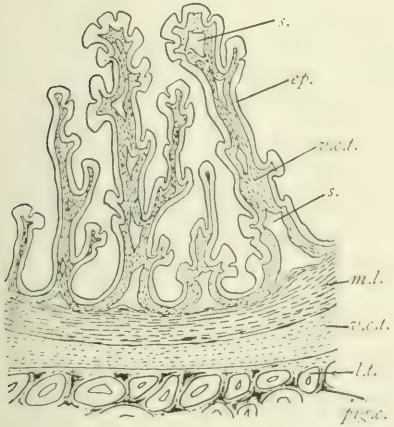


FIGURE 6. — Section of cardiac portion of the stomach, showing the rugæ. *s.*, sinus; *ep.*, epithelial coat; *v. c. t.*, vascular connective tissue; *m. l.*, muscular layer; *l. t.*, liver tubules; *pig. c.*, pigment cells.

As the pyloric end of the stomach is approached the character of the rugæ becomes much more simple, though still adapted to ready absorption. No secreting structures such as the glands of Lieberkühn occur in the stomach, and no digestive fluid of any kind is elaborated by it, according to our experience.

Three large diverticula — the hepatic ducts — open from the stomach into the liver mass, and rapidly divide into smaller ducts, and finally into the minute tubules of that organ. These diverticula can hardly be regarded as mere ducts for the hepatic secretions, since the characteristic enzymes of the liver are not found in the stomach contents to any large extent. They apparently serve as cæca in which the final hydrolysis of fats and carbohydrates largely occurs, and where the products of

this hydrolysis are absorbed and retained.[1] The liver is undoubtedly a secreting organ, as its highly complex glandular epithelium would suggest; but it is equally an organ adapted to digestion, absorption, and retention. A most unique feature of the liver, in this connection, is its function in storing up the metals zinc, copper, and iron. The presence of large quantities of the former two metals in the hepatic tissue has already been noted elsewhere,[2] and a more complete discussion of the liver must be reserved for a later paper. The presence of the zinc and copper seems to have no effect upon the digestive function of the gland with which this paper is more immediately concerned. As will be evident from the experimental data, the liver secretes the enzymes which hydrolyze fats and carbohydrates, viz., a lipase, an amylase, and an invertin. No proteolytic activity could be detected.

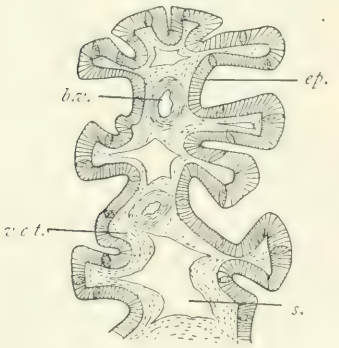


FIGURE 7. — Section of the tip of one of the rugæ more highly magnified, showing the mechanism for rapid absorption. *ep.*, epithelium; *s.*, sinus; *b. v.*, blood-vessel; *v. c. t.*, vascular connective tissue.

The intestine extends from the pyloric sphincter to the anal papilla in the mantle cavity. It is a thin walled tube of simple epithelium, surrounded by a sheath of vascular connective tissue. Absorption may go on here, but only to a limited extent, since the digestive residues are retained in the stomach until the absorption there is complete, whereupon the contraction of the muscular coat of the stomach, and the opening of the sphincter allow the entire mass to be evacuated. No typical digestive enzymes were discovered in the intestinal wall.

Digestion experiments. — In carrying out the digestion experiments upon which the preceding conclusions are based, every attempt was made to exclude as far as possible the influence of disturbing factors, such as putrefaction, bacterial contamination, etc. Control trials were conducted in each case with boiled portions of the various

[1] It is of interest in this connection to note further, that while glycogen is found in abundance in the muscles of this animal, and is undoubtedly the form in which part of the carbohydrate of the food is ingested, none could be obtained from the liver. Dextrose and fats are, however, always present in abundance.

[2] H. C. BRADLEY: Science, N. S. 1904, xix, p. 196.

glandular extracts used; and where doubtful results were obtained, the experiments were repeated. The extracts were made with the organs removed from the living specimens. The usual procedure was to grind the gland with fine sand and extract with a 2 per cent sodium fluoride solution, or in some cases with chloroform-water or glycerin, and filter at once. To follow as closely as possible the conditions obtaining in the actual environment of the animal, all digestions were made at a temperature of about 15°C., and in solutions having the amphoteric reaction of the normal organs.

The first experiment was undertaken to demonstrate the nature and reaction of the salivary secretion. A fresh section of the resting glands reacts slightly alkaline to litmus. For the purpose of obtaining the actual secretion, a capillary cannula was inserted into the salivary duct of a living animal, and electrical stimulation applied to the gland and to the duct itself, — a method which Krause[1] employed successfully in obtaining the salivary secretion of Octopus. The operation was necessarily a difficult one, and only a small amount of the secretion was obtained. It was a clear liquid, ropy from its high content of mucin, and reacted slightly alkaline to litmus.

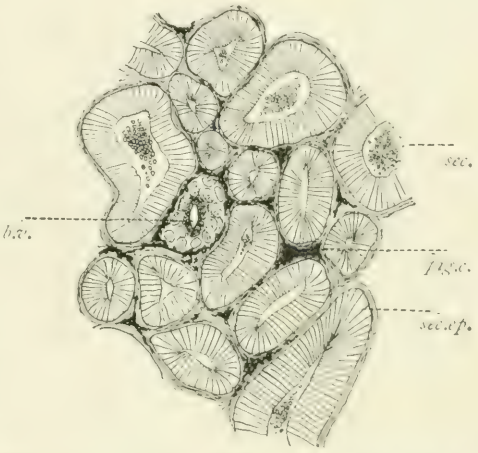


FIGURE 8. — Section of liver tissue, showing the secreting tubules of narrow columnar epithelial cells, surrounded by connective tissue rich in the black pigment cells characteristic of this animal and in which a large part of the copper of the liver is stored. *sec.*, secretion; *pig. c.*, pigment cells; *sec. ep.*, secreting epithelium; *b. v.*, blood-vessel.

Several series of experiments were then undertaken to locate the presence of proteolytic enzymes and kinases activating them.

Series I. — Extracts of the salivary glands, œsophageal mucosa, liver, and intestinal mucosa were made. Inasmuch as the stomachs of many of the specimens were found distended with a digestion mixture, the contents of several were removed, made up with sodium

[1] R. KRAUSE: Centralblatt für Physiologie, 1895, ix, p. 273.

fluoride to a strength of 2 per cent, and tested along with the gland extracts. Further, to discover whether the blood itself contained proteolytic ferments in any quantity, fresh portions of it also were examined. The method used was that devised by Fermi,[1] and employed later by Bayliss and Starling[2] in their studies of pancreatic proteolysis. It consists in subjecting tubes filled with colored gelatin —sterile and rendered antiseptic with sodium fluoride — to the action of the various extracts. The gelatin is liquefied whenever exposed to a solution containing a proteolytic enzyme, and the comparative activities of the various extracts may then be expressed in millimetres of gelatin dissolved, provided the tubes are of the same calibre. The trials were continued for thirty-six hours in the cold (15°C.) with the results summarized below:

TABLE I.

DIGESTION TRIALS WITH GELATIN.

No.	Extract used.	Gelatin dissolved.	No.	Extract used.	Gelatin dissolved.
		mm.			mm.
1	Stomach contents . .	7.5	7	Mixture of 6 + 3 + 4 .	0.0
	Do. boiled	0.0		Do. boiled	0.0
2	Salivary extract . .	15.0[1]	8	Mixture of 1 + 2 . .	16.5
	Do. boiled	0.0		Do. boiled	0.0
3	Stomach mucosa . .	0.0	9	Mixture of 6 + 1 + 2 .	14.0
	Do. boiled	0.0		Do. boiled	0.0
4	Intestinal mucosa . .	0.0	10	Mixture of 2 + 5 . .	12.0
	Do. boiled	0.0		Do. boiled	0.0
5	Liver	0.0	11	Blood	0.0
	Do. boiled	0.0		Do. boiled	0.0
6	Œsophageal mucosa .	0.0			
	Do. boiled	0.0			

[1] A digestion trial made at the same temperature with an active preparation of KÜHNE's dry pancreas powder, showed scarcely more vigorous proteolytic action than did the salivary gland extract used above.

It is evident that the stomach contents and the salivary extract are both proteolytically active; but since the stomach mucosa, the liver, and the mucosa of the œsophagus failed to indicate any proteo-

[1] CL. FERMI: Archiv für Hygiene, 1891, xii, pp. 238–260.

[2] BAYLISS and STARLING: Journal of physiology, 1904, xxx, p. 61.

lytic enzymes, we conclude that the ferment present in the contents of the stomach was merely that of the salivary secretion present with the food.

The method of Fermi may be open to the objection that gelatin is employed, and that different results might be looked for by using a true proteid instead of it. A second series was therefore undertaken to eliminate this possible source of error.

TABLE II.

DIGESTION TRIALS WITH FIBRIN.

No. of trial.	Nature of the extracts.	Amount used.	Dry fibrin used.	"Non-coagulable Nitrogen" in digestion fluids.		Difference.	Nitrogen in tannic acid filtrates.		Difference.
				Unboiled extract.	Boiled extract.		Unboiled extract.	Boiled extract.	
		c.c.	gram	gram	gram	gram	gram	gram	gram
1	Salivary	75	0.692	0.094	0.058	0.036+	0.033	0.024	0.009+
2	Œsophageal	"	"	0.038	0.033	0.005+	0.022	0.024	0.002−
3	Stomach contents	"	"	0.018	0.027	0.009−	0.011	..	..
4	Stomach mucosa	"	"	0.036	0.040	0.004−	0.011	0.019	0.008−
5	Intestinal mucosa	"	"	0.027	0.022	0.005+	..	..	..
6	Liver	"	"	0.045	0.045	0.000	0.021	0.018	0.003+
7	Salivary + intestinal	"	"	0.042	0.000	0.042+	0.022	0.015	0.007+
8	Stomach mucosa + œsophageal mucosa	"	"	0.038	0.040	0.002−	0.019	0.018	0.001+
9	Liver + intestinal mucosa	"	"	0.040	0.036	0.004+	..	..	..
10	Blood	50	"	0.016	0.019	0.003−	..	..	..

Series II. — For these trials extracts with 2 per cent sodium fluoride solution were made as before, using, as nearly as possible, comparable amounts of the tissues, and equal amounts of the solvent. Weighed portions of finely chopped fibrin were added, and the digestions continued for twenty-four hours. At the end of the period all the mixtures were brought to a boil to arrest the action of the ferments, the coagulated proteids filtered off, and a measured portion of the filtrate, representing an aliquot of the original digestion mix-

ture, analyzed for (a) total nitrogen in non-coagulable compounds ; (b) nitrogen in compounds not precipitated by tannic acid, *i.e.*, amido-acids, etc. The actual extent of digestion was ascertained by comparison with figures obtained in a similar way from the boiled (control) extracts. The results are summarized in the table on page 517.

As will be seen from the table, 1 and 7 are the only trials in which the amount of non-coagulable nitrogen exceeds that of the control by a significant amount. (Indeed for the purposes of this comparison, the figures beyond the second decimal place are negligible.) In both of these digestions the extract of the salivary gland is evidently the active factor. There is no indication of the presence of an activating kinase in any of the mixtures, since the simultaneous action of various extracts shows no increased proteolysis.

A salivary extract was allowed to act for forty-eight hours upon fibrin in the cold to determine whether the enzyme produced leucin and tyrosin, under those conditions. As is indicated by the table above, only very small traces of the amido-acids were formed during a twenty-four hour period, and similar results were obtained from the longer digestion. Presumably the products of proteolysis in Sycotypus are absorbed as proteoses and peptones, and scarcely reach the stage of cleavage in which the amido-acids appear. The digestion mixture

TABLE III.

SHOWING THE RELATIVE AMOUNTS OF STARCH PASTE LIQUEFIED IN TRIALS WITH VARIOUS TISSUE EXTRACTS.

(The figures indicate centimetres.)

1	Salivary	0.0	5	Intestinal mucosa .	0.7
2	Œsophageal . . .	0.0	6	Liver	6.0
3	Stomach mucosa . .	0.0	7	1 + 2	0.0
4	Stomach contents .	2.0	8	1 + 6	5.0

gave a strong tryptophan reaction at the end of forty-eight hours, the salivary enzyme resembling trypsin in this respect.

Series III. — In searching for amylolytic enzymes, extracts were made in the same manner as described above. Tubes of a thick starch paste were prepared and subjected to the action of the various extracts and their mixtures, with frequent shaking. As in the case

of the Fermi gelatin tubes, liquefaction of the paste was taken as the indication of digestion. From the nature of the experiment, the figures are only approximate. The trials were continued for five hours, and the control tubes (subjected to boiled extracts) failed to show liquefaction of the starch in any case.

The liver extract is thus seen to contain the active amylolytic enzyme, while the other extracts gave negative results as a rule. The fact that the stomach contents showed a slight starch-digesting power is attributable to the presence of a small amount of the hepatic secretion; the slight degree of activity is rather surprising and indicates that the largest part of the carbohydrate digestion takes place in the hepatic ducts. A further indication of this is to be found in the lack of the hepatic pigments in the stomach contents. As a rule, the digesting mass in the stomach is a colorless, viscid, fluid or semi-fluid material. The contents of the hepatic ducts, on the other hand, are usually colored dark brown from the pigments present in the hepatic secretion. The slight liquefaction seen in No. 5 is due probably to some of the hepatic enzyme retained mechanically from a previously evacuated digestion residue.

In a prolonged starch digestion, the liver extract was found to produce a reducing sugar, which gave an osazone whose crystalline form and melting point (205°) were characteristic of dextrosazone. Thus the liver enzyme of Sycotypus is comparable with the dextrose-forming hepatic enzymes of the livers of higher animals, rather than with ptyalin.

Series IV. — In another series of trials (*cf.* p. 28) a weighed amount of glycogen in solution was substituted for the starch paste, and the amount of reducing sugar formed at the end of the period (and determined by the Allihn gravimetric method for dextrose), was taken as the measure of amylolytic activity. The results confirm those of Series III.

A qualitative experiment was made to determine whether or not the liver extract contained an invertin, with positive results. The formation of invert sugar from saccharose was so obvious that a quantitative determination was not made.

Series V. — Finally the lipase of the liver was demonstrated by a method in common use where comparative rather than quantitative results are desired. A blue litmus-milk emulsion[1] was made, and

[1] This method was first suggested by HEIDENHAIN. *Cf.* ROEHMANN: Chemsche Arbeiten für Mediciner, 1904, p. 53.

to similar portions the various extracts were added. The liberation of free fatty-acids from the neutral milk fats reddens the litmus emulsion; and in this case the results were so distinct that no more accurate method was applied. (*Cf.* Table V.)

TABLE IV.

DIGESTION TRIALS WITH GLYCOGEN.

Extract used.	Amount taken.	Total dextrose (calculated).
	c.c.	gram
Salivary	25	0.09
Salivary boiled . . .	25	0.04
Liver	25	0.72
Liver boiled	25	0.19 [1]
Liver + Salivary . . .	25	0.48

[1] The presence of a reducing sugar in the liver itself is indicated by the boiled control. It also was found to be dextrose.

TABLE V.

FAT-DIGESTION TRIALS.

Extract used.	Amount of extract.	Amount of blue litmus milk.	Color noted in		
			1 hour.	3 hours.	6 hours.
	c.c.	c.c.			
Liver	10	25	Blue	Red	Red
Liver boiled . . .	10	25	Blue	Blue	Blue
Salivary gland . .	10	25	Blue	Blue	Blue
Salivary gland boiled	10	25	Blue	Blue	Blue

SUMMARY. — Digestion in Sycotypus is effected by the secretions from (1) the salivary glands, and (2) the liver or hepato-pancreas.

The salivary glands, closely resembling histologically the comparable glands of higher animals, secrete a ropy mucin-laden solution containing a proteolytic enzyme. This acts normally upon proteids in the cold, and in solutions of a neutral or amphoteric

reaction. In its behavior and in the characteristic decomposition products which it produces, it resembles trypsin.

The liver, a highly glandular organ with very characteristic secreting epithelium, elaborates enzymes which effect the hydrolysis of carbohydrates and fats: an amylase, invertin, and a lipase. The amylase is similar to the characteristic amylolytic enzymes of the vertebrate liver.

Digestion takes place in the stomach proper, and in the so-called hepatic ducts. In the stomach, the proteids are broken down to proteose and peptone, and the products of digestion absorbed through the rugæ. Fats and carbohydrates are largely hydrolyzed in the hepatic ducts, where also the products of their hydrolysis are absorbed and retained.

SOME NOTABLE CONSTITUENTS OF THE URINE OF THE COYOTE.[1]

By ROBERT E. SWAIN.

[*From the Chemistry Laboratory of the Leland Stanford Jr. University.*]

IN the course of an investigation of kynurenic acid as a product of excretion in the urine of the dog, an effort was made to find this interesting substance in the urine of some animal other than the dog, to which it has thus far seemed to be wholly restricted. Various attempts have been made to detect it in the urine of the rabbit[2] and of the cat[3] under conditions of diet which have appeared to be most favorable to its formation, but without success. Hofmeister[4] probably made the first careful research for it in human urine with negative results, and Capaldi[5] failed to find it in the urine of the related animals, the wolf and fox.

Pursuing this line of investigation further, it seemed that good results might come from a study of the urine of the coyote, an animal closely related to the dog, which inhabits the arid districts of the western part of North America.

In general appearance the coyote strongly resembles the dog. It is lean and slender, — weighing from 15 to 30 kilograms, — yellowish gray in color, and combines the cunning and swiftness and the easy, graceful movements of the fox with the greed of the wolf.

[1] The results presented in this paper form a part of a doctoral dissertation on "The formation of kynurenic acid by the animal body," embodying work carried on under the direction of Professor LAFAYETTE B. MENDEL and presented to the faculty of Yale University, May 1, 1904. As this investigation of the urine of the coyote was in a measure only incidental to the subject in question, the results are thus given in a separate paper.

[2] MENDEL and JACKSON: This journal, 1898, ii, p. 1.

[3] MENDEL and JACKSON: *Loc. cit.*

[4] HOFMEISTER: Zeitschrift für physiologische Chemie, 1881, v, p. 67.

[5] CAPALDI: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 87.

It is wary and hard to approach by day, but much bolder at night, when it will frequently venture near to human habitation in committing its depredations, the especial prey being domestic fowls, young lambs, and calves. It shows remarkable cunning in avoiding traps, and is rarely caught in this way, even after every precaution is taken by the trapper.

Until late years it was customary to regard the coyote as a single species ranging over the whole of western North America, from Canada to the table lands of Mexico, and from the western part of the Mississippi Valley to the Pacific Ocean; but in a recent paper Merriam[1] maintains that the name "coyote," as popularly applied, really covers an assemblage of species which comprise three well-marked subordinate groups and a considerable number of distinct geographical forms. According to his classification, the specimen made use of in this investigation belonged to the "Microdon" group, of the form known as *Canis ochropus* Eschscholtz, ranging over the interior valleys and lower foothills of central and southern California. It was a young, but full-grown male weighing 24 kilograms.

The animal was confined in one of the laboratory dog cages, which are arranged for the separate collection of urine and fæces, and fed 250–350 grams of lean meat for several days, until it had become accustomed to its surroundings and to the regular diet. The urine was then collected in daily portions in clean, stoppered bottles, thymol added to prevent bacterial decomposition, and the samples thus preserved for analysis.

Kynurenic acid was found in every urine sample examined. Allantoïn was also present in every case, and in addition to these there appeared a crystalline substance whose composition and properties would go to show it to be some new constituent of carnivorous urine, though it very nearly corresponds with Jaffé's "Urocaninsaüre" in composition and general properties.

Three of the samples collected on succeeding days with the animal on a constant diet of 300 grams of meat and 50 grams of lard gave the following results. Kynurenic acid was determined after the method of Capaldi,[2] and allantoïn according to Loewi's[3] method. The

[1] Merriam: Proceedings of the Biological Society of Washington, D.C., 1897, xi, pp. 19–33.

[2] Capaldi: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 92.

[3] Loewi: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 20.

latter substance was identified by its crystal form and its melting point.

Sample.	Urine volume.	Kynurenic acid.	Allantoïn.
		gram	gram
1	230	0.122	0.031
2	322	0.141	0.074
3	280	0.155	0.062

The remaining samples were combined and a portion used in making a general chemical examination, since the coyote is a representative of a numerous species of carnivorous animal whose urine has probably not heretofore been analyzed.

The total nitrogen was estimated according to the Kjeldahl-Gunning method; urea, by the method of Mörner and Sjöquist (since the presence of allantoïn, according to Mörner,[1] does not materially affect the results); uric acid, by Ludwig's method; allantoïn and kynurenic acid as above.

The following data were obtained as an average of closely agreeing duplicate analyses:

Specific gravity	1.048	
Reaction	Slightly acid.	
Total nitrogen	36.381	grams per litre.
Urea	70.144	" "
Uric acid	0.018	" "
Allantoïn	0.264	" "
Kynurenic acid	0.422	" "
Total sulphuric acid	2.995	" "
Ethereal sulphuric acid	0.127	" "

Allantoïn has previously been found in the urine of suckling calves, occasionally in that of the dog, cat, and in human urine. Much interest has centred in allantoïn as a constituent of animal urine, especially in regard to its origin. That its amount is appreciably increased by the ingestion of food rich in nuclear material, as thymus or pancreas, has been conclusively demonstrated. The main question now at issue is its relation to uric acid. Although it is easily prepared in the laboratory by the gentle oxidation of uric acid by lead

[1] MÖRNER: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 297.

peroxide, mercuric oxide, and other oxidizing agents, its origin from uric acid in the animal body is yet a disputed question.[1]

The high specific gravity of the urine is unusual as an average over so long a period. Doubtless the natural habits of the animal contribute much to this. Its favorite haunts are the arid desert regions of the Pacific slope, where water is very scarce even in winter. In captivity, with fresh water always provided in the cage, it drank surprisingly little. Fat was never touched until the last morsel of lean meat was devoured, and dog cakes, cracker meal, and carbohydrate food in general were not relished and often refused.

During the interval of nearly two months between the time of collection and the analysis of the urine a small crystalline deposit appeared in each sample. An examination under the microscope showed a homogeneous mass of thin, crystalline, hexagonal plates, very similar to those formed by cystin. They slowly dissolved in alcohol and in hot water, but not in ether. On adding sulphuric acid or nitric acid to the hot water solution, there separated beautiful colorless crystals, which differed in crystal form for the two acids, and were also unlike the crystals of the original substance. After washing with water and alcohol until no considerable trace of acid could be detected in the washings, the crystals gave strong tests for sulphuric acid and nitric acid, respectively, and evidently were salts formed by direct union with the acids.

In order to obtain more of the crystals, two litres of the urine were evaporated to small volume on the water bath, strongly acidified with sulphuric acid and allowed to stand forty-eight hours. The precipitate was filtered off and decomposed with hot baryta water. After removing the precipitated barium sulphate, and concentrating the filtrate, about 600 milligrams of colorless crystals separated on standing over night. These were combined with the first yield, the whole recrystallized from hot alcohol (60 per cent) and analyzed. The crystals melted at 208°, and at 220° began to decompose rapidly, as shown by the liberation of considerable carbon dioxide, along with other gases.

The result of the analysis was as follows:

0.1026 gram dried to constant weight at 110° lost 0.0213 gram = 20.86 per cent.

[1] See "Ergebnisse der Physiologie," i, Erste Abteilung, pp. 624–627, for a general discussion of the subject. Also MENDEL and BROWN: This journal, 1900, iii, p. 267; SWAIN: *Ibid.*, 1901, vi, p. 38; MENDEL and WHITE: *Ibid.*, 1904, xii, p. 85; KUTSCHER and SEEMANN: Centralblatt für Physiologie, 1904, xvii, p. 715.

0.1182 gram dried to constant weight at 110° lost 0.0246 gram = 20.81 per cent.

The rest of the substance was dried, combined with the above portions, and analyzed. The following data were obtained:

	I.	II.	Calc. as $C_{12}H_9N_4O_4$
C	52.73	52.60	52.70
H	3.21	3.26	3.33
N	20.69	20.60	20.55
O (by diff.)	23.38	23.53	23.42

The crystalline substance would then have the formula

$$C_{12}H_9N_4O_4 \cdot 4\ H_2O.$$

In many of its properties, and very nearly in its composition, this substance corresponds to a substance found first by Jaffé,[1] and recently by Massot,[2] in the urine of the dog. At the same time it must be admitted that the difference in composition shown by the elementary analysis is sufficient to indicate that this substance is some new constituent of carnivorous urine. Jaffé gave the name "Urocaninsäure" to the substance isolated by him, and estimated its formula to be $C_{12}H_{12}N_4O_4 \cdot 4\ H_2O$. He was unable to find it in the urine of most dogs, and the natural inference has been that it was a casual constituent of this urine which arose either from some metabolic idiosyncrasy of the dog in question, or as a result of nitrototuol feeding, to which the animal had been previously subjected. In regard to the latter possibility, however, Jaffé insists that the dog was perfectly healthy, and that all effects of the nitrototuol feeding had vanished months before. Moreover, other dogs failed to excrete the substance after prolonged administration of nitrototuol.

As soon as another coyote is available, it is the purpose of the writer to isolate more of this substance and to determine its properties more fully.

[1] Jaffé: Berichte der deutschen chemischen Gesellschaft, 1874, vii, p. 1669; and 1875, viii, p. 811.

[2] See Siegfried: Zeitschrift für physiologische Chemie, 1898, xxiv, p. 399.

THE CHEMISTRY OF THE PROTEIN-BODIES OF THE WHEAT KERNEL.[1] PART I.—THE PROTEIN SOLUBLE IN ALCOHOL AND ITS GLUTAMINIC ACID CONTENT.

By THOMAS B. OSBORNE and ISAAC F. HARRIS.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

AS a result of an extended investigation of the proteids of the wheat kernel, Osborne and Voorhees[2] concluded that only one protein substance soluble in alcohol was present in this seed.

Fleurent[3] also held the same view.

Morishima's[4] investigations led him to believe that wheat gluten contained but a single protein, and that the glutenin and gliadin of Osborne and Voorhees were derivatives of one and the same protein substance, which he named artolin.

Ritthausen[5] again asserted his belief in the existence in this seed of three distinct protein-bodies that were soluble in alcohol, but offered no new evidence of their existence.

Kossel and Kutscher,[6] following Ritthausen's directions, prepared the proteins of wheat gluten, and determined the proportion of basic products which they yielded on decomposition with acids. They found that the protein insoluble in alcohol, called by Ritthausen gluten-casein, by Osborne and Voorhees glutenin, was sharply distinguished from the protein soluble in alcohol by the fact that, on

[1] This paper is the first of a series giving the results of an investigation of the nature and amount of the primary decomposition products of the protein constituents of the wheat kernel. The expense of this work has been met by a grant from the Carnegie Institution.

[2] Osborne and Voorhees: American chemical journal, 1893, xv, p. 392.

[3] Fleurent: Comptes rendus de l'académie des sciences, 1896, cxxiii, p. 755.

[4] Morishima: Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 348.

[5] Ritthausen: Journal für praktische Chemie, 1899, lix, p. 474.

[6] Kossel and Kutscher: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 165.

decomposition, it yields a notable quantity of lysine; whereas all their products derived from the alcoholic extract of the gluten yielded *none* of this diamino acid. This fact shows conclusively that Morishima's view of the identity of all the protein-products obtained from wheat gluten is incorrect.

They further found that Ritthausen's so-called mucedin, gliadin, and gluten-fibrin, prepared from the alcoholic extract of wheat gluten, yielded somewhat different proportions of histidine and arginine; but, in view of the methods employed for the determination of these bases, they considered these differences too small to fully justify the conclusion that these were distinctly different protein substances. They conclude their paper, however, with the statement that their experiments confirm the views of Ritthausen respecting the composition of wheat gluten.

Kutscher[1] extended the investigation of these proteins, and determined in the solutions which remained from Kossel and Kutscher's determinations of the bases the amount of tyrosine and glutaminic acid.

As a result of this work, he concluded that mucedin and gliadin were one and the same protein; but that the gluten-fibrin yielded an amount of glutaminic acid so much less than that given by the other fractions as to leave no doubt that this was a distinctly different substance.

Nasmith[2] concluded that only one alcohol-soluble protein was present in wheat gluten. He also stated that, although this protein, gliadin, invariably contains phosphorus, it is not a nucleo-proteid. In passing, we may say that this statement is erroneous, for an examination of a large number of our preparations has shown them to be wholly free from phosphorus.

Very recently König and Rintelen[3] have published a brief account of the results of an investigation which they have made to determine the nature of the proteids of wheat gluten, and conclude, with Ritthausen, that there are three present which are soluble in alcohol.

As a result of all these later investigations, it would appear that glutenin is sharply distinguished from the alcohol-soluble protein by the presence of lysine among its decomposition products, and that,

[1] Kutscher: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 111.

[2] Nasmith: Transactions of the Canadian Institute, 1903, vii.

[3] König and Rintelen: Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, 1904, viii, p. 401.

notwithstanding the close agreement in ultimate composition, these two proteins do not have a common origin, as Morishima supposed. Respecting the existence of more than one protein soluble in alcohol, all recent investigators, except Kossel and Kutscher, and König and Rintelen, agree with the view formerly advanced by the writer.

For the purpose of this investigation of the decomposition products yielded by the proteins of the wheat kernel, it has become necessary, as the first step, to examine carefully the new evidence which has been presented respecting the existence of Ritthausen's gluten-fibrin and mucedin.

The first difficulty encountered lay in the impossibility of following Ritthausen's directions for preparing the several alcohol-soluble proteins which he has named, since many evidently important details are omitted in the description of these methods.

Kossel and Kutscher state that their products were made according to these directions, but give no details, nor do they state which method they employed. Kutscher concludes his paper by the statement that "the wheat gluten consists of gluten-casein wholly insoluble in cold 60 per cent alcohol; gluten-fibrin, but little soluble, and gliadin, easily soluble in cold 60 per cent alcohol."

Although we have made a very large number of preparations representing fractions of this protein substance, dissolved by alcohol of various degrees of strength, we have never obtained any that were not either completely soluble in cold alcohol of 60 per cent, by volume, or else contained such insignificant quantities which did not dissolve, that we have found it impossible to make from them a preparation of "gluten-fibrin" suitable for further examination. We have, therefore, been unable to repeat the work of Kossel and Kutscher and are entirely at a loss to understand how their preparation of "gluten-fibrin" was obtained.

König and Rintelen describe their procedure in more detail. These investigators extracted wheat gluten with absolute alcohol, added ether to the alcoholic extract, and united the precipitate produced with the extracted gluten. This latter was then extracted with 65 per cent alcohol, and to the extract alcohol was added until the mixture contained 88–90 per cent of alcohol.

After decanting from the precipitate that had formed, the solution was filtered clear and evaporated to dryness on the water bath, finely pulverized, and extracted with ether, to remove fat. As all of the fat could not be thus removed, the mass was again dissolved in alcohol,

to which some caustic potash was added, and this solution shaken out several times with ether. The weakly alkaline solution was then exactly neutralized with hydrochloric acid, and evaporated on the water bath. The product thus obtained was their "gluten-fibrin."

The precipitate produced by 88–90 per cent alcohol was washed with alcohol of the same strength and dissolved in a little 65 per cent alcohol. From this, one-half of the alcohol was distilled off and the residual solution cooled, when a precipitate separated. From this the solution was decanted, leaving a mass of gliadin. The solutions which remained from several such precipitates were united and distilled, until one-third of the solvent was removed. On cooling the residual solution, a deposit formed which they considered to be a mixture of gliadin and mucedin. The solution decanted from this deposit was evaporated to dryness, and yielded a considerable residue of "mucedin."

The analyses of these products showed that the gliadin thus prepared had the same composition as that obtained by us, as well as that made by Ritthausen, while the "gluten-fibrin" and mucedin contained about 1 per cent less nitrogen and much more carbon.

That the "gluten-fibrin" thus made by repeated evaporation at a high temperature with water, and solution in caustic alkali, can represent an unaltered constituent of the wheat kernel, seems to us highly improbable, especially in view of the fact that gliadin contains a very large proportion of amide nitrogen; *i. e.*, about one-fourth of its total nitrogen, which would probably be easily split off by caustic alkalies. Furthermore, although gliadin can be heated in strongly alcoholic solutions without apparent change, when heated in aqueous solutions a not inconsiderable part is altered.

We have never attempted to produce "gluten-fibrin" by such a process as here described, as we are convinced that products thus prepared cannot certainly be regarded as unaltered constituents of the wheat kernel. We have, however, many times isolated the proteid soluble in the strongest alcohol; but although these preparations have represented very small fractions of the total protein under examination, and have been obtained from solutions in very strong alcohol, they have always shown the same properties and composition as the other purified preparations of gliadin.

König and Rintelen obtained their mucedin from the nearly aqueous solutions remaining after separating the gliadin by evaporating to dryness. We assume that the residue which remained was

subjected to some further purification, but concerning this they say nothing. We have always found that this solution contained many impurities, and that when the protein substance in it had been properly purified, this had the properties and composition of gliadin.

We have recently made large quantities of gliadin, and subjected it to very careful and extensive fractionation, but, as in the earlier work of Osborne and Voorhees, we have been wholly unable to obtain any evidence whatever of the existence of "mucedin."

In this work we have used only the purest absolute alcohol, which we have ourselves prepared in large quantities, in order to avoid the presence of acids and other substances which might be otherwise introduced. It is possible that the American wheats which we have examined differ from the European, but this we consider in the highest degree improbable. Furthermore, it would seem improbable that our gliadin could be contaminated by "gluten-fibrin" and "mucedin," which we certainly did not succeed in separating from it, and, at the same time, show so close an agreement in composition with that of König and Rintelen, from which they suppose that both of these proteins had been carefully removed. It is also improbable that Kjeldahl should have found $(a)_D$ uniform for successive fractional precipitations of the alcohol-soluble protein, if the material which he examined was a mixture of three different substances, nor, if this were the case, could his determination of $(a)_D$, -92°, be expected to agree so closely with ours. -92.3°.

The composition of both "gluten-fibrin" and "mucedin" differs from that of gliadin just as one would expect if these former substances were slightly altered, and somewhat impure products obtained from gliadin.

Until more convincing evidence of the existence of "gluten-fibrin" and "mucedin" as distinct protein substances is brought forward, we cannot consider them to be original constituents of the wheat kernel.

As fractional precipitation has wholly failed, in our hands, to yield products similar to the "gluten-fibrin" and "mucedin" of Ritthausen, we have sought to discover, if possible, differences in the amount of glutaminic acid which our different preparations might yield, and so be able to judge of the value of the evidence presented by Kutscher respecting the existence of gluten-fibrin.

Experimental Part.

Two portions of different preparations of gliadin, each weighing 100 grams, were decomposed by boiling for fourteen hours with 200 c.c. of concentrated hydrochloric acid. The solution, cooled with ice, was saturated with gaseous hydrochloric acid and, packed in ice, kept in a refrigerator for three days.

The entire solution, thus treated, solidified to a thick mass of crystals which were sucked out with a pump, thoroughly washed with ice-cold alcohol which, according to a suggestion made to us by P. A. Levene, had been saturated with hydrochloric acid. When dried over sodium hydrate, the two preparations thus obtained weighed, respectively 55.15 and 58.33 grams. The filtrate and washings, when concentrated to a syrup, yielded by a repetition of the preceding process a second crop of crystals, weighing, respectively, 4.91 and 2.68 grams, making the total crude glutaminic acid in each case 60.06 and 60.01 grams. Since gliadin, when decomposed by boiling with acids, yields 4 per cent of nitrogen as ammonia, these products were examined for ammonium chloride. By crystallizing one of them from strong alcohol 9.72 grams of ammonium chloride, and 34.75 grams of nearly pure glutaminic acid hydrochlorate were obtained. As considerable loss of glutaminic acid occurred during this process, the other product was treated dry with absolute alcohol, and the residue washed thoroughly with absolute alcohol, dried, and found to weigh 5.64 grams.

The solution and washings were concentrated to a syrup, taken up in water and freed from color by animal charcoal. The aqueous solution was then evaporated to small volume, and concentrated hydrochloric acid added in quantity. It was then evaporated until crystallization began. On cooling, the solution set to a solid mass of large crystals, which was sucked out with a pump and washed with ice-cold alcoholic hydrochloric acid. When dried over caustic soda, these crystals, fraction *a*, weighed 39.12 grams. The filtrate and washings from *a*, when concentrated, yielded 2.15 grams of crystals, *b*, which examination showed to be nearly pure ammonium chloride, and the filtrate from this 1.42 grams, *c*, which was likewise ammonium chloride. Fractions *b* and *c* were united and dried at 100°. 0.3370 gram distilled with MgO gave ammonia equal to 8.64 c.c. of HCl. 1 c.c. HCl = 0.0100 gram N = 25.63 per cent N. Calculated for NH_4Cl, 26.22 per cent.

The solution from which *a*, *b*, and *c* had been separated, was then boiled with a small excess of barium hydrate, to remove the remaining ammonia, and the barium removed with an equivalent quantity of sulphuric acid.

On concentration with an excess of hydrochloric acid, this solution yielded *d*, weighing 4.43 grams, and the filtrate from *d*, on long standing on ice, gave 2.41 grams of *e*.

A part of fraction *a* was then distilled with magnesia. 0.6815 gram, dried at 100°, gave ammonia equal to 1.34 c.c. HCl. 1 c.c. HCl = 0.0100 gram N. It gave ammonia, therefore, equal to 7.51 per cent of NH_4Cl. 0.6541 gram of fraction *a* dried at 100°, and treated according to Kjeldahl, neutralized 5.9 c.c. HCl. 1 c.c. HCl = 0.0100 gram N. It contained, therefore, 9.02 per cent of N. Deducting the ammonium chloride thus found, the remaining 92.49 per cent of fraction *a* contains 7.62 per cent of N. Calculated for $C_5H_9NO_4HCl$, 7.64 per cent.

From the results of this analysis, we find that fraction *a* contained 36.18 grams of glutaminic acid hydrochlorate. Fractions *d* and *e* were pure glutaminic acid hydrochlorate, as shown by their crystalline form and nitrogen content.

Fraction *d*. 0.5544 gram dried at 100°, treated according to Kjeldahl, neutralized 4.32 c.c. of HCl. 1 c.c. HCl = 0.0100 gram N, equal to 7.79 per cent N. Calculated for $C_5H_9NO_4HCl$, 7.64 per cent.

Fraction *e*. 0.5092 gram dried at 100° gave, according to Kjeldahl, ammonia equal to 3.86 c.c. HCl. 1 c.c. HCl = 0.0100 gram N, equal to 7.58 per cent. Calculated for $C_5H_9NO_4HCl$, 7.64 per cent.

The amount of the glutaminic acid hydrochlorate thus found, was as follows:

	grams.
In fraction *a*	36.18
In fraction *d*	4.43
In fraction *e*	2.41
	43.02

This is equivalent to 34.42 grams of free glutaminic acid.

Since the gliadin decomposed contained 7 per cent of moisture, the amount of glutaminic acid thus found was equal to 37 per cent of the dry gliadin. This may be taken as a minimal figure, for the true proportion is certainly higher, as some glutaminic acid remained in the mother liquors and could not be separated in a pure state.

In confirmation of these figures, this determination was repeated with two fractions of the alcohol-soluble protein of wheat gluten, which had been separated from relatively strong alcoholic solutions and should, therefore, have contained a large proportion of "gluten-fibrin," if the statements respecting the solubility of this substance are correct.

Two portions of different preparations of the air-dry substance, equivalent to 18.62 and 14.65 grams dried at 110°, were hydrolized as before, freed from ammonia by evaporating with an excess of baryta, and from barium by an equivalent quantity of sulphuric acid. The solution was then decolorized with animal charcoal, and evaporated with an excess of hydrochloric acid until crystallization began. The glutaminic acid hydrochlorate which separated, when washed with ice-cold alcoholic hydrochloric acid and dried, weighed, respectively, 8.69 and 6.27 grams, equivalent to 37.33 and 34.2 per cent of free glutaminic acid in the protein.

Another attempt was made to isolate a fraction of "gluten-fibrin" by dissolving 200 grams of a preparation, representing the total alcohol-soluble protein of wheat gluten, in a mixture of 900 c.c. of absolute alcohol and 600 c.c. of water; that is, in alcohol of 60 per cent by volume. Although the solution was somewhat turbid, nothing was deposited, even on long standing. We then added 300 c.c. of water to the solution, making the alcohol 50 per cent; but still nothing separated. The strength of the alcohol was, therefore, raised to 75 per cent, by adding 1800 c.c. of absolute alcohol, and a very large precipitate, *A*, at once separated. By adding 2000 c.c. of absolute alcohol to the clear solution from which *A* separated, another large precipitate, *B*, was produced, and in the filtrate from *B* a third precipitate, *C*, resulted, when a further large quantity of absolute alcohol was added. This last product weighed 20 grams, and constituted only 10 per cent of the total protein. The solution from which *C* separated contained only traces of protein, and *C*, therefore, represented the fraction of the whole protein soluble in the strongest alcohol, and should, consequently, contain much "gluten-fibrin." In 17.5 grams of this substance, dried at 110°, we next determined the glutaminic acid produced by decomposing with hydrochloric acid. By proceeding in the same manner as in the experiment last described, we isolated 7.8914 grams of pure glutaminic acid hydrochlorate, which is equivalent to 6.2131 grams of the free acid, or 35.50 per cent.

0.7296 grams, dried at 110°, gave ammonia equal to 5.66 c.c. HCl (1 c.c. HCl = 0.0100 gram N) = 7.75 per cent N. Calculated for $C_5H_9NO_4HCl$ = 7.64 per cent N.

This result seems to furnish conclusive evidence that there is no fraction, soluble in very strong alcohol, to be obtained from the alcohol-soluble protein of wheat gluten that is characterized by yielding a relatively small proportion of glutaminic acid.

Since Kutscher decomposed his proteins with sulphuric acid, while we used hydrochloric acid in the preceding experiments, we made the following experiment, in order to determine whether the higher yield obtained by us might not be due to this fact. We accordingly boiled, for fourteen hours, with a mixture of 150 grams of sulphuric acid and 300 c.c. of water, 50 grams of one of the preparations of gliadin from which we had previously isolated 37 per cent of glutaminic acid.

The resulting solution was treated with an excess of baryta, and the ammonia expelled by evaporation. The barium was then removed by an equivalent amount of sulphuric acid, and the filtered solution evaporated. Some tyrosine separated, which was filtered out, and the evaporation continued until the volume was quite small. On standing, an abundant quantity of crystals of free glutaminic acid separated, and from the mother liquor, by further concentration and standing, a second crop of crystals was obtained. After recrystallizing several times, 8.48 grams of pure glutaminic acid was obtained, which contained 9.43 per cent of nitrogen.

0.6094 gram substance gave, according to Kjeldahl, ammonia equal to 5.75 c.c. HCl (1 c.c. HCl = 0.0100 gram N) = 9.43 per cent. Calculated for $C_5H_9NO_4$, 9.48 per cent. From the mother liquors which yielded this glutaminic acid, we further separated, by saturating with hydrochloric acid, and proceeding in the manner already described, 4.055 grams of well crystallized hydrochlorate which contained 7.73 per cent of nitrogen.

0.4983 gram substance gave ammonia equal to 3.85 c.c. HCl (1 c.c. HCl = 0.0100 gram N) = 7.73 per cent N. Calculated for $C_5H_9NO_4HCl$ = 7.64 per cent N.

The free glutaminic acid corresponding to this amount of the hydrochlorate is 3.244 grams, making a total of 11.724 grams. Since the 50 grams of air-dry gliadin were equivalent to 46.33 grams, dried at 110°, this amount of glutaminic acid is equal to 25.3 per cent, or about the same proportion as Ritthausen found in his preparation of

"mucedin," after decomposing with sulphuric acid, but much more than the 19.81 per cent found by Kutscher in the same substance. The amount, however, was only about two-thirds as much as we found after decomposing with hydrochloric acid, and although the separation was not complete, there was no reason to suppose that more remained in the mother liquors in one case than in the other.

In conclusion, we here bring together the results of these determinations, that they may be more readily compared:

PERCENTAGE OF GLUTAMINIC ACID YIELDED BY GLIADIN

	decomposed by HCl				H_2SO_4
Prep.	1	2	3	4	5
	37.00	37.33	34.2	35.50	25.3

All these are minimal figures, since in each case some glutaminic acid still remained in the mother liquors, but we do not think that more than relatively insignificant quantities were lost, unless in the first separation from the mass of decomposition products. Respecting the amount which escaped separation from this solution, we know absolutely nothing.

From these results it would appear:

1. That Kutscher's determinations of glutaminic acid fall far short of the actual quantity of this substance yielded by the alcohol-soluble protein of wheat, and that they, therefore, afford no evidence which justifies the conclusion that this substance consists of two distinct protein bodies.

2. That fractional precipitations of this alcohol-soluble protein yield practically the same large proportion of glutaminic acid, so that, in view of their very close agreement in composition and properties, both physical and chemical, we have every reason to believe that only one such protein is present, for which, we think, the name gliadin should be retained.

3. That gliadin yields a remarkable proportion of glutaminic acid, much in excess of that from any other known protein, and greater than that of any single decomposition product yet obtained in a pure state from any other true protein substance, the protamines, of course, excepted.

4. That this very large proportion of glutaminic acid in a food protein, so extensively used, is a matter of great importance in relation to the food value of this substance, and deserves further careful study.

APPROXIMATELY COMPLETE ANALYSES OF THIRTY "NORMAL" URINES.

By OTTO FOLIN.

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Total nitrogen. — Kjeldahl's method was applied as follows: 5 c.c. of urine are measured out by means of small burettes (with glass stopcocks) graduated in twentieths of a cubic centimetre, 20 c.c. sulphuric acid, and a small quantity of copper sulphate + potassium sulphate are used for the decomposition, the boiling being continued about one-half hour. The normal acid and alkali from which the tenth normal solutions are obtained, are prepared with sodium bicarbonate (made from sodium) as control. "Alizarin red" is used as indicator. Numerous duplicate determinations have convinced me that this procedure gives perfectly reliable results with urine.

It may be permissible to point out in this connection that the conversion of the urinary nitrogen into ammonia by means of sulphuric acid involves very little oxidation. It is essentially a hydrolytic reaction, and not an oxidation.[1]

Urea.[2] — The method used in this laboratory is as follows: 5 c.c. of urine are measured into an Erlenmeyer flask (capacity, 200 c.c.), 5 c.c. concentrated hydrochloric acid, 20 gm. crystallized magnesium chloride, a piece of paraffine the size of a small hazelnut, and finally two or three drops aqueous, 1 per cent solution of "alizarin red" are added. The safety tube described in my second paper on the subject is then inserted, and the mixture boiled until each returning drop from the safety tube produces a very perceptible bump. The heat is then reduced somewhat, and the heating is continued for a full hour.

Particular attention must be called to the addition of the indicator

[1] Malfatti: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 469; Folin: *Ibid.*, 1904, xli, p. 239.

[2] Folin: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 504; 1902, xxxvi, p. 333; 1903, xxxvii, p. 548; Mörner, K. A. H.: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 297.

alizarin red, as its use in this combination has not been described before. This indicator is so stable as to withstand the heat, and it turns bright red in the presence of the smallest trace of alkali. The boiling contents in the Erlenmeyer flask must not be allowed to remain alkaline, and as soon as they begin to turn red, a few drops (but only a few) of the acid distillate in the safety tube are shaken back into the flask. At the end of an hour the contents of the flask are put into a litre flask with about 700 c.c. water and 20 c.c. 10 per cent sodic hydrate, and the ammonia distilled off. This distillation must be continued until the contents of the litre flask are nearly dry, or till the distillate shows no trace of ammonia with delicate litmus paper (about one hour). The distillate is then boiled a few minutes to drive off the carbonic acid, cooled and titrated in the usual manner. Alizarin red is used as indicator. It is very serviceable in all cases where moderate quantities of ammonium salts are present.

This procedure is believed to give highly accurate and reliable results. The combination of this method with the Mörner-Sjöquist method, as described by Mörner, *loc. cit.*, must be used with urines containing sugar, because when carbohydrates and urea are heated together they combine, according to Schoorl[1] into very stable condensation products, the ureids. Sugar was absent in the urines here investigated. I have, however, several times applied the combination of the two methods, but with practically identical results.

The highest degree of accuracy is of course the most important point in connection with any analytical method, but as long as scientific investigations are still largely limited to more or less isolated individual efforts, it will be necessary at times to sacrifice the last degree of accuracy for the sake of saving time. The extraordinarily careful work of Mörner would seem to indicate that the combination method which he used was a trifle more accurate than my method taken by itself. I freely acknowledge that this may well be the case, and that Mörner's urea values represent the highest degree of accuracy at present obtainable. The only reason why I hesitate to accept this conclusion as quite beyond question is the fact that the accuracy of Mörner's figures obtained by the combination method depends upon the accuracy of his ammonia determinations. The latter were made according to Schlösing, but the important details upon which the

[1] Schoorl, N.: Chemisches Centralblatt, 1903, lxxiv, p. 1079.

accuracy of Schlösing's method depends cannot be said to have been at all well-known prior to the publication of Shaffer's paper[1] on the subject in 1903. The method is at best only moderately accurate, and if used according to the directions given in textbooks of physiological chemistry and urine analysis, the results are far from correct. Leaving sugar urines out of consideration, it is therefore not quite excluded that the small differences in the results of the two procedures may at least in part be due to inaccuracies in the ammonia-determinations. The fact that duplicate Schlösing determinations were made, furnishes very little additional guarantee of accuracy even in the hands of such a skilled and careful investigator as Mörner. Be this as it may, and accepting the differences which Mörner found as being in favor of the combination method, they are in the case of normal urines so small (most frequently no difference at all) that I have not hesitated to continue the use of the urea-determinations as described above. The saving of time and attention is of no small importance when a consecutive series of urines are to be as completely analyzed as has here been the case, and the combination method used by Mörner requires much more time, as well as more continuous attention than the simple method here used.

Ammonia.[2] — The ammonia is set free by the addition of a weak alkali (sodic carbonate), is then removed from the urine at ordinary room-temperature by means of a strong air current, collected in tenth normal acid, and titrated.

25 c.c. of urine is measured into an aerometer cylinder (30–45 cm. high), and about a gram of dry sodic carbonate and some crude petroleum (to prevent foaming) are added. The upper end of the cylinder is then closed by means of a doubly perforated rubber stopper, through which pass two glass tubes, only one of which is long enough to reach below the surface of the liquid. The shorter tube (about 10 cm. in length) is connected with a "calcium chloride tube" filled with cotton, which in turn is connected with a glass tube extending to the bottom of a wide mouth bottle (capacity about 500 c.c.) which contains tenth normal acid (20 c.c.), water (200 c.c.), and indicator for the absorption of the ammonia. The complete absorption of the ammonia is most easily insured by the use of a simple but effective absorption tube which compels a very intimate

[1] SHAFFER: This journal, 1903, viii, p. 331.

[2] FOLIN: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 161; SHAFFER, this journal, 1903, viii, p. 330.

contact of the air coming from the cylinder with the acid and water in the absorption bottle. For a description of this tube the reader is referred to the original paper (*loc. cit.*, p. 169). It is easily made, but it can also be obtained from Eimer and Amend (New York). The air passing first through the alkaline urine, and then through the standard acid, transfers every trace of ammonia in the course of a relatively short time ($1\frac{1}{2}$ hours) from the urine to the acid, and is then determined by direct titration.

For the accuracy of this method I can vouch. The air current must, however, be rapid (several hundred litres per hour). An ordinary good filtering pump, or better an inverted filtering pump, a blast pump, is sufficient if the water-pressure is not too low.

Kreatinin.[1] — The principle upon which this determination is based is the color-reaction given by kreatinin, and by no other normal urinary constituent, with picric acid in an alkaline solution. In order to make the color-comparisons accurate enough for quantitative purposes a high grade colorimeter is necessary. The French instrument of Duboscq, obtained through Eimer and Amend, is eminently satisfactory. Its construction and workmanship are excellent, the prisms are perfectly flawless, and the height of each solution can easily be adjusted to within an accuracy of a tenth of a millimetre.

Half-normal potassium bichromate containing 24.55 gm. per litre, saturated picric acid solution containing about 12 gm. per litre, and 10 per cent sodic hydrate solution are the reagents needed.

10 c.c. urine is measured into a 500 c.c. volumetric flask, 15 c.c. picric acid and 5 c.c. sodic hydrate are then added, and the mixture is allowed to stand for five to six minutes. This interval is used to pour a little of the bichromate solution into each of the two cylinders of the colorimeter. The depth of the solution in one of the cylinders is then accurately adjusted to the 8 mm. mark. With the solution in the other cylinder a few preliminary colorimetric readings are made simply for the sake of insuring greater accuracy in the subsequent readings of the unknown solution. The two bichromate solutions must of course be equal in color, and in taking their readings no two should differ more than 0.1 mm. or 0.2 mm. from the true value (8 mm.), leaving out of consideration the very first reading made, which is sometimes less accurate. Four or more read-

[1] Folin: Zeitschrift für physiologische Chemie, 1904, xli, p. 223.

ings should be made in each case, and an average taken of all but the first. After a while one becomes very sure of the true point, and can take the average of the first two readings.

At the end of five minutes the contents in the 500 c.c. flask are diluted up to the 500 c.c. mark. The bichromate solution is thoroughly rinsed out of one of the cylinders by means of the unknown solution and several colorimetric readings are then made *at once.*

The calculation of the results is very simple. If, for example, it is found that it takes 9.5 mm. of the unknown urine-picrate solution to equal the 8 mm. of the bichromate, than the 10 c.c. of urine contains $10 \times \frac{8.1}{9.5} = 8.4 +$ mg. kreatinin.[1]

The amount of urine taken for the determination is usually 10 c.c., but if this should be found to contain more than 15 mg. or less than 5 mg. kreatinin, the determination should be repeated with a correspondingly different amount of urine, because outside of these limits the determination is much less accurate. With kreatinin solutions, the results are uniformly surprisingly accurate, and I have as yet found no reason for believing that the method is not equally reliable, at least for normal urines. The color of the urine does not materially affect the result on account of the very great dilution.

A kreatinin-determination can be made by this method in less than fifteen minutes.

Uric acid.[2] — The uric acid determinations are carried out as follows:

The chief reagent is a solution of 500 gm. ammonium sulphate, 5 gm. uranium acetate, and 60 c.c. 10 per cent acetic acid in 650 c.c. water.

150 c.c. urine is measured into a tall, narrow beaker, or a cylinder, and $37\frac{1}{2}$ c.c. of the reagent is added (if the available quantity of urine is not too small, 200 c.c. urine and 50 c.c. reagent are used). The mixture is allowed to stand without stirring for about half an hour. The uranium precipitate has then settled and the clear supernatant liquid is removed by siphoning or by decantation. 125 c.c.

[1] The calculation is based on the experimentally determined fact that 10 mg. of perfectly pure kreatinin, give under the conditions of the determination 500 c.c. of a solution, 8.1 mm. of which has exactly the same colorimetric value as 8 mm. of half-normal bichromate solution.

[2] FOLIN and SHAFFER: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 552.

of this liquid is measured into another beaker, 5 c.c. strong ammonia is added, and the mixture set aside until the following day. The precipitate is then filtered off, washed with 10 per cent ammonium sulphate solution until the filtrate is quite or nearly free from chlorides. The filter is removed from the funnel, opened, and the precipitate rinsed back into the beaker. Enough water to make about 100 c.c. is added, and finally the precipitate is dissolved by means of 15 c.c. concentrated sulphuric acid, and at once titrated with $\frac{n}{20}$ potassium permanganate solution, each c.c. of which corresponds to 3.75 mg. uric acid. A correction of 3 mg., due to the solubility of the ammonium urate, is added to the result. The very first pink coloration, extending through the entire liquid from the addition of two drops permanganate solution, while stirring with a glass rod, marks the end point of the titration.

Chlorides. — The chlorides are titrated according to Volhard, and the only point that need be mentioned is the fact that a fair amount of nitric acid must be added in order to get a clear filtrate.

Phosphates. — The phosphates are determined volumetrically by means of uranium acetate solution, using powdered potassium ferrocyanide as indicator. Chemically pure crystallized monopotassium-phosphate is used to standardize the uranium solution.[1]

Total sulphur and sulphates. — A few general remarks concerning sulphate precipitations in urine may not be unnecessary. To get correct results, it is indispensable that the barium chloride be added very slowly to the hot acidified urine. If the barium is added too rapidly the precipitate will invariably carry down other salts which the subsequent washings with hot water will not remove. To illustrate :

Urine A, 0.50 c.c. + 25 c.c. $BaCl_2$, added in about thirty seconds gave 0.2759 gm. and 0.2780 gm. barium sulphate. The same urine, with the barium chloride added by means of a capillary funnel (made from a calcium chloride tube) so constructed as to deliver 25 c.c. in five to seven minutes, gave 0.2713 gm. and 0.2693 gm. barium sulphate.

Urine B. gave under similar conditions by the first procedure 0.4032 gm. and 0.4052 gm. barium sulphate, and by the latter procedure only 0.3897 gm. and 0.3900 gm. barium sulphate.

Among the sulphur constituents of urine there are very minute

[1] See SUTTON's Volumetric Analysis, 8th ed., p. 316.

quantities of substances which can scarcely be determined separately, but which are included in the total sulphur, and which must therefore be counted as inorganic, ethereal, or as neutral sulphur. Such substances are traces of hydrogen sulphide very frequently present in urine, and the sulphocyanides which are probably always present. In the scheme of analysis here followed, these substances are included in the ethereal sulphates, not because they belong there, but it is most convenient not to exclude them in the ethereal sulphate determinations. This arrangement has in a measure proved unfortunate, because of the unexpected importance which the ethereal sulphates have acquired in the course of these investigations, an importance which was not at all foreseen at the time the analytical scheme was adopted. That the errors in the ethereal sulphate determinations due to this fact are not important enough to vitiate the conclusions drawn, is shown by the following determinations made with and without the use of potassium chlorate. The analyses were made in urines representing the eighth, ninth, and tenth days of a starch and cream diet described in the next paper.

Urine	A,	133	c.c.	with	the use of $KClO_3$	gave	45.8	mg. $BaSO_4$.
"	"	"		without	"	"	37.6	" "
"	B	"		with	"	"	43.8	" "
"	"	"		without	"	"	34.7	" "
"	C	"		with	"	"	41.1	" "
"	"	"		without	"	"	34.1	" "

Inorganic sulphates.[1] — To 50 c.c. of urine in a (200 c.c.) Erlenmeyer flask are added 5 c.c. of a 4 per cent potassium chlorate solution and 5 c.c. concentrated hydrochloric acid. When this mixture has boiled a few minutes (5–10) it is perfectly colorless. The capillary dropping funnel (described above) is then inserted, and the barium chloride (25 c.c. 10 per cent solution) is poured into the funnel. After a few minutes, the heat is reduced to below the boiling point, and the mixture kept at this temperature for one-half to one hour. The barium sulphate is washed for half an hour with hot water, and at intervals of a few minutes hot ammonium chloride solution (5 per cent) is substituted for the water, so that in all five or six additions of ammonium chloride take place in the course of the first twenty minutes of the washing. Filter-paper and

[1] Folin: This journal, 1902, vii, p. 152.

precipitate are then at once partially dried by folding and pressing gently between dry filter-papers, and are then transferred to the weighed porcelain crucible. 3 or 4 c.c. of alcohol are poured into the crucible and ignited. This dries and partially burns the filter-paper, and the residue is then burned to whiteness, cooled and weighed. The weight of the ethereal sulphate precipitate, separately determined, is subtracted from the result.

Ethereal sulphates. — 200 c.c. of the urine, previously diluted to 1 litre if necessary, is measured into a beaker, and 100 c.c. barium chloride solution (10 per cent) added in the cold. The mixture is set aside for twenty-four hours, and the clear supernatent liquid poured into a second dry beaker by decantation. This preliminary decantation is necessary, because the barium sulphate precipitate will otherwise go through the filter. The decanted liquid is filtered, and 150 c.c. of the clear filtrate, corresponding to 100 c.c. of urine, is measured into an Erlenmeyer flask (capacity 400 c.c.), 10 or 15 c.c. concentrated hydrochloric acid, and 10–15 c.c. 4 per cent potassium chlorate are added, and the mixture is heated to boiling. The remainder of the operation is similar to that described for the inorganic sulphate determination.

Total and neutral sulphur. — The total sulphur is determined in the urinary residue burned in the presence of 0.3–0.5 gm. potassium carbonate and previously titrated for the "mineral acidity." It may seem questionable whether the urinary residue can be burned in the presence of such small quantities of alkali without loss of sulphur. The oxidation is, however, in this case a slow process, and is therefore free from the dangers that prevail when the carbonate-nitrate fusion mixture is used. A series of comparative determinations made to test the accuracy of the method have shown the results to be very reliable, and incidentally have shown that the ignition with the fusion mixture does not give correct results unless large quantities are used. This is shown by the following figures:

Ignition with K_2CO_3.	Ignition with Fusion Mixture.
0.297 gm. = 0.1366 gm. $BaSO_4$	About 3 gm. = 0.1335 $BaSO_4$
0.377 gm. = 0.1365 gm. "	" 3 gm. = 0.1358 "
0.291 gm. = 0.1370 gm. "	" 6 gm. = 0.1372 "
0.342 gm. = 0.1366 gm. "	" 6 gm. = 0.1372 "
	" 10 gm. = 0.1375 "
	" 9 gm. = 0.1390 "

If the mineral-acidity determinations are not to be made, the nitrate-fusion-mixture method, or better still, the sodium peroxide method, is the more convenient; but where such acidity determinations are made, it would be useless loss of time to ignite a separate sample of urine for the sulphate determinations. (For details of evaporating and igniting the urine with small quantities of carbonate alone, see the mineral-acidity determination.)

From the total sulphate as obtained by this process are subtracted the inorganic and the ethereal sulphates, in order to get the "neutral" sulphur of the urine.

Indican. — The indican-determinations recorded in this paper will need a somewhat fuller explanation, because the method used for recording these values is wholly arbitrary. Exactly one-hundredth of a twenty-four-hour quantity of urine is taken for each determination. In this the indigo is developed by the addition of Obermeyer's reagent, and the indigo-blue taken out by means of 5 c.c. chloroform. With the chloroform solutions are then made colorimetric comparisons, using Fehling's solution as a standard. The Fehling's solution is given the arbitrary value of 100.

The advantage of this method is that it takes very little time, and the standard for comparison is one that is kept on hand in every laboratory.

It has been my intention to work out an exact method on this principle, by using the same colorimeter as is used in the kreatinin-determinations for the color comparisons, and then finding the value of Fehling's solution in terms of pure indigo. The difficulties in the way of such a colorimetric method are by no means small, the chief of which is to produce a pure blue solution from the indican without any admixture of red coloring matter.

The method as described gives sufficiently definite values to enable any one to repeat the experiment, and get comparable values; but it does, of course, in the present form, not possess the accuracy that would entitle it to be classed as quantitative.

Total Acidity.[1] — To 25 c.c. of urine are added 15–20 gm. powdered potassium oxalate, and one or two drops of a 1 per cent phenolphtalein solution. The mixture is shaken rapidly for one or two minutes, and titrated at once, *i. e.*, while still cold from the effects of the dissolved oxalate, until a faint but unmistakable tint remains permanent on further shaking.

[1] FOLIN: This journal, 1903, ix, p. 265.

Mineral acidity or the excess of mineral acids or bases.[1] — Bunge,[2] Magnus-Levy,[3] Soetbeer,[4] and many others have studied the balance of the acid and basic forming elements in urine by means of separate determinations of all the different metals and acids. Here the same result is obtained by direct titration of the burned urinary residue.

To 25 c.c. of urine in a platinum dish is added from 0.3 to 0.5 gm. K_2CO_3, weighed within an accuracy of two-tenths of a milligram. The solution is evaporated to dryness, and the residue ignited, when perfectly dry, over a radial burner using at first a very low heat, and at no time allowing the dish to become more than faintly red hot. The dish is heated at this temperature for one hour, then cooled, and 10 c.c. of hydrogen peroxide is added and evaporated. The dried residue is ignited as before for one hour. It is dissolved in an excess of tenth normal hydrochloric acid and water (50–75 c.c. $\frac{n}{10}$ HCl), transferred to an Erlenmeyer flask, boiled to remove carbonic acid, and cooled. One or two drops phenolphtalein solution and a few crystals of neutral potassium oxalate (to precipitate the calcium) are added, and the solution is titrated as usual. The ammonia, the acidity of the hydrogen peroxide, and the acidity of the organic sulphur (neutral and ethereal, 8 gm. of which are taken to represent 1 c.c. tenth normal acid) must be subtracted from the result given by the direct titration. These values, as well as the acidimetric value of the potassium carbonate, must be separately determined.

This procedure gives very reliable results, if proper care is used in the evaporation and the burning of the urine. It is to be used only when the actual excess of mineral acids above that necessary for the neutralization of the mineral bases is to be estimated, or when the total amount of organic acids in urine (whether free or combined with bases) is to be determined.

For the determination of the *free* mineral and organic acidity, titrate the total acidity as described above, then determine the phosphates, and subtract their acidimetric value (1 c.c. tenth normal acid for each 7.1 mg. P_2O_5) from the total acidity. The remainder is the acidity due to uncombined organic acids, and the difference,

[1] Folin: This journal, 1903, ix, p. 270.

[2] Bunge: Physiologie des Menchen, 1901, p. 420.

[3] Magnus-Levy: Archiv für experimentelle Pathologie und Pharmakologie, 1899, xxxxii, p. 149.

[4] Soetbeer: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 96.

that obtained from calculating all the phosphoric acid as diacid phosphate, is the free mineral acidity. If the acidity calculated from the total phosphates is greater than the titrated acidity, then there are practically no free organic acids present, and the titrated acidity represents the amount of phosphates present in the diacid form. Urines of the latter kind are turbid, unless they are also practically free from calcium.

A more detailed discussion of the acidity of urine will be given in the next paper, entitled Laws governing the Chemical Composition of Urine.

II. The Composition of "Normal" Urine Corresponding to a "Standard Diet."

Almost every textbook on physiology and on physiological chemistry gives a sample table to illustrate the composition of normal human urine. Most English textbooks give the table formulated by Parkes a generation ago, and all textbooks that I have had the opportunity to examine cite the same kind of tables now as were cited twenty years ago, *i. e.*, before the introduction of Kjeldahl's method for determining nitrogen. All omit the total nitrogen which the tables are supposed to represent. Yet it was by the help of Kjeldahl's method that Pflüger in 1886 made the important discovery that urea constitutes only about 87 per cent of the total nitrogen, and it is only on the basis of the total nitrogen that percentage studies of the different nitrogenous constituents of urine are possible.

In view of these facts, and in view of the fact to which Bunge has called attention in the different editions of his textbook, that in all the voluminous urine literature there is still no record of the complete analysis of any one concrete sample of a normal twenty-four-hour quantity of urine, I present in Tables I–VII the records of thirty fairly complete analyses.

In Schäfer's textbook of physiology (Vol. I, p. 573) Hopkins, in commenting on two analyses of Bunge's, points out that no great importance can be attached to such collective quantitative analyses of urine unless the diet itself has been simultaneously analyzed. The thirty urines represented by Tables I–VII were obtained from six different normal persons, all kept for seven days on one standard uniform diet; and in order that all the urines should fairly represent the diet, the first two twenty-four-hour quantities were discarded and only the last five were analyzed.

TABLE I—Mr. J. H. B.

Weight in kilos. / Date. Sp.e	Sp. gr. 1.0—. / Volume of urine. c.c.	Total nitrogen. N_2.	Urea. gm. / Urea-N.	Ammonia c.c. $\frac{1}{10}NH_3$. / Ammonia-N.	Kreatinin. gm. / Kreatinin-N.	Uric acid. gm. / Uric acid-N.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
								Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
57.4	17	16.8	28.9	049.0	1.54	0.33	0.71	86.3	5.4	91.7	3.4	0.7	4.2
7	1980		13.5	0.91	0.57	0.11							
	19	16.9	31.7	68.4.0	1.47	0.20	0.49	87.6	5.7	93.3	3.2	0.6	2.9
8	1840		14.8	0.96	0.55	0.10							
	20	17.2	32.5	583.0	1.00	0.36	0.46	88.2	4.8	93.0	3.9	0.7	2.4
9	1655		15.2	0.82	0.58	0.12							
	17	16.3	30.6	580.0	1.56	0.34	0.50	87.7	5.0	92.7	3.6	0.7	3.0
10	1770		14.3	0.81	0.58	0.11							
57.4	16	15.9	29.8	540.0	1.70	0.34	0.50	87.5	4.8	92.3	4.0	0.7	3.0
11	1815		13.9	0.76	0.63	0.11							
Average	1812	16.6	30.6	608.0	1.57	0.33	0.53	87.5	5.1	92.6	3.6	0.7	3.1
			14.3	0.85	0.59	0.11							

TABLE II—Mr. E. H. S.

67.0	27	13.9	25.9	493.0	1.41	0.19	0.53	87.0	4.9	91.9	3.8	0.5	3.8
29	1120		12.1	0.69	0.53	0.06							
	18	15.4	28.7	543.0	1.50	0.26	0.75	87.1	4.8	91.9	3.6	0.6	4.9
30	1740		13.4	0.76	0.56	0.09							
	22	14.5	26.7	480.0	1.49	0.20	0.70	85.8	4.7	90.5	3.8	0.5	5.2
1	1260		12.5	0.68	0.55	0.07							
	20	14.3	25.8	475.0	1.44	0.22	0.92	84.5	4.7	89.2	3.8	0.6	6.4
2	900		12.1	0.67	0.54	0.07							
66.1	32	15.9	29.7	465.0	1.42	0.33	0.73	87.3	4.1	91.4	3.3	0.7	4.6
3	960		13.9	0.65	0.53	0.11							
Average	1196	14.8	27.3	492.0	1.46	0.24	0.72	86.2	4.6	90.8	3.6	0.6	5.0
			12.8	0.69	0.54	0.08							

TABLE I — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" S_3. "S_3."	In per cent of Total Sulphur. S_1.	S_2.	S_3.	Acidity in c.c. $\frac{n}{10}$. Total, titrated.	Mineral.	Organic.	Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
7	3.40	3.04	0.18	0.18	89.4	5.3	5.3	515	..	..	3.62	7.3	10	
8	3.56	3.19	0.20	0.17	89.6	5.6	4.8	574	140	434	3.73	6.5	70	
9	3.40	3.06	0.19	0.15	90.0	5.6	4.4	550	298	252	3.57	6.1	85	
10	3.42	3.00	0.19	0.23	87.7	5.5	6.8	580	205	375	3.79	5.7	80	
11	3.27	3.00	0.18	0.09	91.7	5.5	2.8	552	174	378	3.76	5.8	12	
Av'ge	3.41	3.06	0.19	0.17	89.6	5.5	5.0	554	204	360	3.69	6.3	51	

TABLE II — *Continued.*

29	3.08	2.64	0.23	0.21	85.7	7.4	7.0	605	398	207	3.61	6.8	90	
30	3.09	2.62	0.25	0.22	84.8	8.1	7.1	668	403	265	3.86	6.5	90	
1	3.08	2.64	0.24	0.20	85.7	7.3	7.0	680	378	302	3.74	5.3	90	
2	3.09	2.65	0.27	0.17	85.7	8.7	5.5	698	435	265	3.84	5.1	80	
3	3.22	2.83	0.29	0.10	87.9	9.0	3.1	695	472	223	4.06	5.9	80	
Av'ge	3.11	2.67	0.25	0.19	85.8	8.0	6.1	669	417	252	3.82	5.9	85	

TABLE III—Mr. R. L. J.

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Sept.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
70.8	*19*		*29.3*	*466.0*	*1.64*	*0.24*							
21	1520	15.9	13.7	0.64	0.61	0.08	0.81	85.9	4.1	90.0	3.8	0.5	5.7
	21		*31.0*	*514.0*	*1.56*	*0.30*							
22	1530	16.6	14.5	0.72	0.58	0.10	0.80	86.9	4.3	91.2	3.6	0.6	4.6
	22		*30.6*	*520.0*	*1.53*	*0.33*							
23	1460	16.6	14.4	0.73	0.56	0.11	0.83	86.5	4.4	90.9	3.4	0.7	5.0
	21		*30.4*	*532.0*	*1.67*	*0.37*							
24	1430	16.5	14.2	0.75	0.52	0.12	0.90	86.1	4.5	90.6	3.2	0.7	5.5
70.1	*23*		*30.7*	*618.0*	*1.45*	*0.33*							
25	1380	16.6	14.5	0.86	0.54	0.11	0.85	85.7	5.2	90.9	3.3	0.7	5.1
			30.3	*529.0*	*1.57*	*0.31*							
Av'ge	1444	16.4	14.1	0.74	0.59	0.10	0.85	86.2	4.5	90.7	3.5	0.6	5.3

TABLE IV—Mr. G. E. C.

70.0	*18*		*30.6*	*571.0*	*1.88*	*0.42*							
31	1740	16.2	14.3	0.80	0.70	0.14	0.30	87.9	4.9	92.8	4.3	0.9	2.0
Aug.-Sept.	*24*		*28.0*	*542.0*	*1.74*	*0.41*							
1	1220	15.0	13.1	0.76	0.65	0.14	0.48	86.6	5.0	91.6	4.3	0.9	3.2
	26		*25.8*	*485.0*	*1.67*	*0.36*							
2	1010	13.9	12.0	0.68	0.62	0.12	0.41	86.7	4.9	91.6	4.5	0.9	3.0
	20		*27.4*	*606.0*	*1.72*	*0.36*							
3	1330	14.8	12.8	0.85	0.64	0.12	0.39	87.0	4.7	91.7	4.3	0.8	3.1
71.1	*29*		*25.8*	*539.0*	*1.82*	*0.62*							
4	910	14.0	12.0	0.75	0.68	0.21	0.36	85.4	5.4	90.9	4.9	1.5	2.7
			27.4	*549.0*	*1.77*	*0.44*							
Av'ge	1242	14.8	12.8	0.77	0.66	0.15	0.41	86.7	5.0	91.7	4.5	1.0	2.8

TABLE III — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
21	3.31	2.85	0.25	0.21	86.1	7.6	6.3	589	219	370	3.98	6.3	140	
22	..	3.00	0.25	..	..	..	..	630	299	331	4.16	5.7	150	
23	3.35	2.89	0.28	0.18	86.3	8.3	5.4	625	432	193	3.84	5.8	140	
24	3.20	2.73	0.24	0.13	85.3	7.5	4.1	617	..	..	3.68	5.7	140	
25	3.25	2.92	0.21	0.12	89.8	6.5	3.7	646	276	370	3.85	5.2	130	
Av'ge	3.28	2.88	0.25	0.16	84.7	7.6	4.8	621	308	313	3.90	5.7	140	

TABLE IV — *Continued.*

31	3.22	2.87	0.24	0.11	89.1	7.4	3.4	619	257	362	4.09	6.8	14	
1	3.10	2.76	0.22	0.12	89.0	7.1	3.9	697	410	287	4.10	5.8	85	
2	..	2.67	0.17	..	..	..	..	638	412	226	3.86	5.2	85	
3	3.00	2.69	0.17	0.14	89.7	5.7	4.6	648	388	260	3.94	5.5	80	
4	3.11	2.80	0.18	0.13	90.0	5.8	4.2	691	408	283	3.82	4.6	87	
Av'ge	3.11	2.76	0.19	0.13	89.4	6.5	4.1	601	375	284	3.96	5.6	70	

TABLE V — MR. M. H.

Weight in kilos.	Sp. gr. 15°.	Total nitrogen. N_g.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Aug.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_g.
56.6	18	14.4	27.6	362.0	1.31	0.48	0.37	89.3	3.4	92.7	3.5	1.1	2.9
24	1040		12.9	0.51	0.50	0.16							
25	20	16.4	31.5	470.0	1.38	0.52	0.31	90.0	4.0	94.0	3.7	1.0	2.0
	1590		14.7	0.66	0.51	0.17							
26	145	13.9	26.5	352.0	1.22	0.35	0.46	89.1	3.6	92.7	3.3	0.9	3.3
	2000		12.4	0.49	0.45	0.12							
27	10	15.2	28.9	392.0	1.38	0.47	0.43	89.4	3.6	93.0	3.3	1.0	2.8
	1695		13.5	0.55	0.51	0.16							
56.5	175	15.8	30.2	386.0	1.50	0.50	0.46	89.1	3.4	92.5	3.5	1.1	2.9
28	1930		14.1	0.53	0.56	0.17							
Average	1645	15.1	28.9	392.0	1.36	0.46	0.41	89.4	3.6	93.0	3.3	1.0	2.7
			13.5	0.55	0.50	0.15							

TABLE VI — MR. S. R. B.

57.8	285	16.6	31.3	428.0	1.53	0.44	0.67	88.1	3.6	91.7	3.4	0.9	4.0
13	1065		14.6	0.60	0.57	0.15							
Sept.													
14	275	19.5	36.9	443.0	1.60	0.45	0.90	88.3	3.2	91.5	3.1	0.8	4.6
	1230		17.2	0.62	0.60	0.15							
15	255	18.0	34.7	345.0	1.55	0.48	0.56	90.1	2.7	92.8	3.2	0.9	3.1
	1290		16.2	0.48	0.58	0.16							
16	28	19.4	37.1	511.0	1.64	0.46	0.62	89.3	3.5	92.8	3.1	0.8	3.3
	1240		17.3	0.72	0.61	0.15							
58.2	27	17.7	33.9	438.0	1.60	0.40	0.56	89.4	3.4	92.8	3.3	0.8	3.1
17	1370		15.8	0.61	0.60	0.13							
Average	1239	18.2	34.7	433.0	1.58	0.44	0.66	89.0	3.3	92.3	3.2	0.8	3.7
			16.2	0.60	0.59	0.15							

TABLE V — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur. S_1.	S_2.	S_3.	Acidity in c.c. $\frac{n}{10}$. Total, titrated.	Mineral.	Organic.	Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
24	2.98	2.74	0.16	0.08	91.9	5.4	2.7	499	120	379	2.95	6.8	25	
25	3.31	2.95	0.23	0.13	89.1	7.2	3.7	686	311	375	3.78	7.1	20	
26	2.83	2.51	0.19	0.13	88.7	6.7	4.5	552	316	236	3.26	6.5	3	
27	3.46	3.15	0.20	0.11	91.0	5.8	3.2	613	226	387	3.73	7.1	10	
28	3.65	3.14	0.17	0.34	86.0	4.6	9.4	672	255	417	3.76	7.0	3	
Av'ge.	3.25	2.90	0.19	0.16	89.2	5.8	5.0	604	247	359	3.44	6.9	12	

TABLE VI — *Continued.*

13	3.77	3.38	0.18	0.21	89.6	4.8	5.6	618	264	354	4.07	5.3	95	
14	3.74	3.30	0.26	0.18	88.2	7.0	4.8	674	310	364	4.37	6.1	100	
15	3.63	3.15	0.29	0.19	86.8	8.0	5.2	542	150	392	4.39	6.6	110	
16	..	3.55	0.39	..	..	..	..	768	367	401	5.01	5.5	110	
17	3.80	3.19	0.33	0.28	84.0	8.7	7.3	652	..	..	4.66	7.0	110	
Av'ge.	3.73	3.25	0.25	0.19	87.2	7.2	5.5	651	273	378	4.50	6.1	105	

TABLE VII.

AVERAGES OF TABLES I–VI.

Initial weight, kilos. / Table No.	Volume of urine, c.c.	Total nitrogen, N_2.	Urea. gm. / Urea-N.	Ammonia. c.c. $\frac{n}{10}$ NH_3. / Ammonia-N.	Kreatinin. gm. / Kreatinin-N.	Uric acid. gm. / Uric acid-N.	Undetermined nitrogen.	In per cent of Total Nitrogen.					
								Urea.	Ammonia.	Urea + Ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
57.4 / 1	1812	16.6	30.6 / 14.3	608 / 0.85	1.57 / 0.59	0.33 / 0.11	0.53	87.5	4.1	92.60	3.6	0.7	3.10
67.0 / 2	1196	14.8	27.3 / 12.8	492 / 0.69	1.46 / 0.54	0.24 / 0.08	0.72	86.2	4.6	90.80	3.6	0.6	5.00
70.8 / 3	1444	16.4	30.3 / 14.1	529 / 0.74	1.57 / 0.59	0.31 / 0.10	0.85	86.2	4.5	90.70	3.5	0.6	5.30
70.9 / 4	1242	14.8	27.4 / 12.8	549 / 0.77	1.77 / 0.66	0.41 / 0.15	0.41	86.7	5.0	91.70	4.5	1.0	2.80
56.6 / 5	1645	15.1	28.9 / 13.5	302 / 0.55	1.36 / 0.50	0.46 / 0.15	0.41	89.4	3.6	93.00	3.3	1.0	2.70
57.8 / 6	1239	18.2	34.7 / 16.2	433 / 0.60	1.58 / 0.59	0.41 / 0.15	0.66	89.0	3.3	92.30	3.2	0.8	3.70
Final average.	1430	16.0	29.8 / 13.9	500 / 0.70	1.55 / 0.58	0.37 / 0.12	0.60	87.5	4.3	91.85	3.6	0.8	3.75
Minimum	1196	14.8	27.3 / 12.8	302 / 0.55	1.36 / 0.50	0.24 / 0.08	0.41	86.2	3.3	90.70	3.2	0.6	2.70
Maximum	1812	18.2	34.7 / 16.2	608 / 0.85	1.77 / 0.66	0.46 / 0.15	0.85	89.4	5.0	92.60	4.5	1.0	5.30

TABLE VII — *Continued.*

Table No.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks. Wt.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
1	3.41	3.06	0.19	0.17	89.6	5.5	5.0	554	204	360	3.69	6.3	51	
2	3.11	2.67	0.25	0.19	85.8	8.0	6.1	669	417	252	3.82	5.9	85	
3	3.28	2.88	0.25	0.16	84.7	7.6	4.8	621	308	313	3.90	5.7	140	
4	3.11	2.76	0.19	0.13	89.4	6.5	4.1	601	375	284	3.96	5.6	70	
5	3.25	2.90	0.19	0.16	89.2	5.8	5.0	604	247	359	3.44	6.9	12	
6	3.73	3.25	0.25	0.19	87.2	7.2	5.5	651	273	378	4.50	6.1	105	
														kilos.
Av'ge	3.31	2.92	0.22	0.17	87.8	6.8	5.1	617	304	313	3.87	6.1	77	63.4
Min.	3.11	2.67	0.19	0.13	84.7	5.5	4.1	554	204	252	3.44	5.6	12	56.6
Max.	3.73	3.25	0.25	0.19	89.6	8.0	6.1	669	417	378	4.50	6.9	140	70.9

The diet which these urines represent was made up as follows:

Whole milk	500 c.c.
Cream (18–22 per cent fat)	300 c.c.
Eggs (white and yolk)	450 gm.
Horlick's Malted Milk	200 gm.
Sugar	20 gm.
Sodium chloride	6 gm.

Water enough to make the whole up to two litres.
Extra water to drink 900 c.c.

The ingredients combined into a liquid mixture contained 119 gm. protein and approximately 148 gm. fat and 225 gm. carbohydrates. The following determinations made at different times show that the mixture as prepared had a very constant composition:

Cl_2	SO_3	P_2O_5	N_2
6.25	3.69	5.75	18.92
6.02	3.71	5.54	18.80
	3.62	5.92	18.86
	3.81	5.92	18.86
	3.80		19.05
	3.84		19.05
			19.02
			19.02
			19.02

The above diet fulfils sufficiently well the requirements of the so-called dietary standards, considering the class of persons for whom it was originally intended (patients in a private hospital for the insane). In regard to the protein, it corresponds almost exactly with the value demanded by Voit for a man weighing 70 kgm.; and since four out of the six normal persons experimented upon weighed less than 70 kgm. and the other two weighed no more than 70 kgm., the diet may be said to be liberal rather than too scanty with respect to its protein-content.

It is not my intention to devote any space to a discussion of these thirty normal urines, for the very reason that they correspond so closely to what we have been accustomed to consider normal.

A number of points could profitably be touched upon on the basis of these tables, as, for example, the quantitative relation between ethereal sulphates, neutral sulphur, and indican; between ammonia,

total nitrogen, and the acidities; between chlorides, total nitrogen, and the volume of urine. These and several other more important points can, however, be considered to better advantage on the basis of the experimental material given in the next paper. In order to avoid unnecessary repetitions they may therefore be passed over here. The importance of Tables I–VII lies in the fact that they constitute concrete examples representing the prevailing views concerning the composition of normal human urine.

LAWS GOVERNING THE CHEMICAL COMPOSITION OF URINE.

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I. Introduction.

THE term normal urine as ordinarily used, and as defined by the percentage tables in textbooks, seems to me to be largely a tribute to Voit. The dietary standards of Voit, demanding about 118 gm. protein per day in the food, will invariably give, in normal persons, urines of approximately the percentage-composition represented in the tables of the preceding paper, the variations being confined chiefly to uric acid, chlorides, phosphates, and sulphates, these depending on the corresponding variations in the quality of the food. The meaning ordinarily given to the term normal urine implies, therefore, a tacit acceptance of Voit's dietary standards as substantially correct. Numerous analyses of the urines from people representing different conditions of life and different social strata have shown that very great variations occur, but throughout the voluminous literature on this subject there is to be noted a strong general desire or tendency to corroborate the findings of Voit. In most investigations made of certain nationalities, as, for example, the Japanese, or of certain groups of people, as, for example, vegetarians and some rural populations, there are to be noted more or less strained attempts to show either that such people come nearer Voit's standards than had been supposed, or that they are not properly nourished.

This paper is devoted to a study of human urines obtained from diets which are as different from the so-called standards of diet as they could be made, and the results are believed to furnish a more or less new point of view from which to examine those standards. Further, the analytical data and the generalizations recorded in this paper seem to require a reconsideration of the current theories concerning the nature of protein-metabolism. A discussion of the nature of protein-metabolism will be presented in a later paper, and in connection with it dietary standards will be discussed.

II. The Distribution of the Urinary Nitrogen among Urea, Ammonia, Uric Acid, and Kreatinin.[1]

Few persons probably would be willing to call the urines given in Table I normal, yet they were obtained from an apparently perfectly healthy man who gained rather than lost in weight during the experiment, and who, according to his own statement, consumed, while the experiment lasted, neither less nor appreciably different food from that which he had been consuming during the preceding three years.

One exception must be made to the last statement. Dr. van Someren was not restricted in diet, the purpose being to discover what peculiarities, if any, could be found in his urines while on his ordinary mixed diet (consisting largely of vegetables, bread, crackers, cream, and candies). When the analyses of the first two days' urines had shown that it was indeed a case of a very unusual urine, Dr. van Someren was however induced to try the diet described on page 64, but taking only one-half the quantity there given. This lasted only two days, January 30–31. Even half the quantity of the standard nitrogen-rich diet produced, it will be seen, quite a change in the composition of the urine, the change tending to make it much more like the urines shown in Tables I–VII. It may be added that Dr. van Someren lost in weight on the nitrogen-rich diet, and that he again began to gain as soon as he returned to his own mixed diet.

[1] For the opportunity to examine the urines given in Table I (the starting point of all subsequent experiments recorded in this paper), I am indebted, on the one hand, to Professor Bowditch, and on the other hand, to Dr. Ernest van Someren of Venice.

Professor Bowditch kindly brought Dr. van Someren on a visit to this laboratory, and the latter while here consented to remain the guest of the McLean Hospital long enough to permit the collection of a series of consecutive twenty-four-hour quantities of urine.

Dr. van Someren is known to many readers of this journal through his close association with Mr. Horace Fletcher, a popular writer on the value of the thorough mastication of all kinds of food.

No attempt will be made to discuss in detail the figures for the different urinary constituents shown in the table, as this can be done to better advantage on the basis of the next four feeding experiments, planned especially to bring out in other persons the same characteristics as are to be found in Table I. A few salient points concerning the nitrogenous constituents may, however, be indicated. From Table VII of the preceding paper it will be seen that the average total urinary nitrogen obtained from the protein-rich diet is 16 gm., with a minimum of 14.8 gm. and a maximum of 18.2 gm., and this is certainly what must be demanded from a normal urine of an average-sized man, *if* the Voit or the Atwater dietary standards are to be the measure of what constitutes normal diets. As against this, we find in the above table only from 4.8 gm. to 8 gm. total nitrogen, and the latter figure only from a diet to which Dr. van Someren submitted under protest. Looking at the distribution of the nitrogen as shown by these two tables, we find in the former that on the protein-rich diet the urea represents from 86.3 per cent to 89.4 per cent, or again the values which have been considered normal ever since they were first discovered by Pflüger. But in the latter table the urea-nitrogen has sunk to 62 per cent of the total, and at no time rises above 80.4 per cent. With such a decided fall in the per cent of nitrogen represented by urea in Dr. van Someren's urines, we must of course look for an increase in the per cent of one or more or all of the other nitrogenous constituents. A glance at Table I will show that this is indeed the case. To facilitate comparison, the figures taken from Table VII of the preceding paper and Table I are here placed together.

	Table VII.	Table I.
Total nitrogen	14.8–18.2 gm.	4.8– 8.0 gm.
Urea-nitrogen	86.3–89.4%	62.0–80.4 %
Ammonia-nitrogen	3.3– 5.1 "	4.2–11.7 "
Kreatinin-nitrogen	3.2– 4.5 "	5.5–11.1 "
Uric acid-nitrogen	0.5– 1.0 "	1.2– 2.4 "
Undetermined nitrogen	2.7– 5.3 "	4.8–14.6 "

The wide variations in the composition of Dr. van Someren's urines are chiefly due to the temporary change in diet on January 30 and 31 mentioned above.

The urines recorded in Table I constitute, as far as I know, the first instance in which a urine obtained from a normal man was shown to contain but 62 per cent of its total nitrogen in the form of urea, and on the other hand over 11 per cent of the nitrogen in the form of

kreatinin. The cause of this remarkable transposition of the nitrogen compounds was naturally sought for in the fact that the total amount of protein-metabolism was clearly reduced almost to a minimum.

In the metabolism literature is to be found considerable evidence tending to show that the percentage composition of urine is not the same on a diet containing but little protein as on one rich in that constituent. The experiments of Sivén [1] and of Burian and Schur,[2] showing that the uric acid is to a large extent independent of the total amount of nitrogen eliminated, point in this direction, though the influence of this constituent is of course necessarily very small. The experiments of Gumlich [3] and of Camerer [4] and Pfaundler have shown that the urea-nitrogen is less in per cent of total nitrogen on a vegetarian diet than on a meat diet, although the difference noted by these investigators was not very great (about 80 per cent urea-nitrogen on a vegetarian diet, as against 90 per cent on a meat diet). The full significance of these variations, or the possibility of obtaining much greater differences in the percentage composition of the urinary nitrogen, was clearly not understood by these investigators, or they would have planned some more decisive experiments. Accordingly we find that their results have not noticeably influenced the generally prevailing views concerning the normal composition of human urine. This is very apparent, for example, in the writings of von Jaksch on the distribution of the nitrogen in urines obtained in different diseases. As a final conclusion derived from his numerous investigations, von Jaksch [5] stated only last year (1903) that "urea is and will remain the chief nitrogenous product in the urine of the sick," as of normal persons, *i. e.*, representing from 83 to 91 per cent of the total nitrogen. Von Jaksch goes so far as to assert in a very positive manner that in all metabolism-experiments undertaken in the future for clinical purposes, direct urea-determinations may well be dispensed with, because the correct urea-contents of such urines can be found by simply multiplying the total nitrogen of the urine by the factor 2! Such a rule, to be correct, would require that 93.3 per cent of the total nitrogen in the urine be there as urea! That Camerer, who for many years has

[1] Sivén: Skandinavisches Archiv für Physiologie, 1901, xi, p. 308.

[2] Burian and Schur: Archiv für die gesammte Physiologie, 1901, lxxxvii, p. 239.

[3] Gumlich: Zeitschrift für physiologische Chemie, 1892, xvii, p. 19.

[4] Camerer: Zeitschrift für Biologie, 1903, xlv, p. 1.

[5] Jaksch: Zeitschrift für klinische Medizin, 1903, l.

TABLE I.

DR. E. v. S. (*Normal person*).

Weight in kilos. / Date. Jan.–Feb.	Sp. gr. 1.0— / Volume of urine. c.c.	Total nitrogen. N_2.	Urea. gm. / Urea-N.	Ammonia. c.c. $\frac{n}{10}$ NH_3. / Ammonia-N.	Kreatinin. gm. / Kreatinin-N.	Uric acid. gm. / Uric acid-N.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
								Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
56.6 27	*19* 620	4.2	*5.6* 2.6	*352.0* 0.49	*1.27* 0.47	*0.16* 0.05	0.59	62.0	11.7	73.7	11.1	1.2	14.0
28	*18* 970	4.4	*6.6* 3.1	*283.0* 0.40	*1.13* 0.42	*0.16* 0.05	0.44	70.5	9.0	79.5	9.5	1.1	9.9
29	*20* 820	5.2	*8.9* 4.1	*226.0* 0.32	*1.28* 0.47	*0.20* 0.07	0.19	79.8	6.1	85.9	9.1	1.3	3.7
30	*23* 745	6.1	*9.9* 4.6	*185.0* 0.25	*1.27* 0.47	*0.36* 0.12	0.64	76.2	4.2	80.4	7.0	2.0	10.6
31	*25* 720	8.0	*13.7* 6.4	*302.0* 0.42	*1.11* 0.41	*0.30* 0.13	0.60	80.4	5.3	85.7	5.2	1.6	7.5
1	*21* 670	6.8	*11.8* 5.5	*215.0* 0.30	*1.05* 0.40	*0.33* 0.11	0.44	81.3	4.4	85.7	5.7	1.6	7.0
2	*20* 840	6.8	*11.4* 5.4	*218.0* 0.31	*1.02* 0.38	*0.40* 0.13	0.57	79.2	4.5	83.7	5.5	2.0	8.5
3	*16* 1015	5.1	*8.4* 3.8	*162.0* 0.23	*1.22* 0.45	*0.35* 0.12	0.54	74.2	4.1	78.3	8.8	2.3	10.6
4	*15* 750	4.6	*7.2* 3.4	*282.0* 0.40	*1.22* 0.45	*0.25* 0.08	0.31	72.8	8.6	81.4	9.8	1.8	7.0
58.5 5	*15* 900	4.9	*7.1* 3.3	*252.0* 0.35	*1.17* 0.43	*0.36* 0.12	0.70	67.1	7.1	74.2	9.0	2.4	14.4

TABLE I — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
27	0.73	0.42	0.09	0.22	57.5	12.3	30.2	243	—126	369	1.2	3.9	20	
28	0.87	0.50	0.09	0.28	57.5	10.4	32.1	260	19	241	1.3	2.1	20	
29	1.21	0.65	0.14	0.42	53.7	11.6	34.7	199	—298	497	1.3	3.9	40	
30	..	1.18	0.13	..	..	..	..	209	—48	257	1.6	4.2	35	
31	1.65	1.38	0.14	0.13	83.6	8.4	8.0	363	61	301	2.0	3.3	35	
1	1.26	0.98	0.14	0.14	77.7	11.1	11.0	241	—64	305	1.6	2.5	15	
2	1.11	0.76	0.15	0.20	68.5	13.5	18.0	161	—363	524	1.5	3.4	5	
3	0.91	0.60	0.12	0.19	65.9	13.2	20.9	110	—188	298	0.9	3.8	15	
4	0.86	0.55	0.17	0.14	63.9	19.8	16.3	230	—45	275	1.0	2.3	10	
5	1.04	0.63	0.12	0.29	60.6	11.5	27.9	158	—220	378	0.9	2.9	10	

been engaged in the study of this problem, has not appreciated the significance of some of his own experimental results is definitely shown in his last article on the subject published in 1903.[1] Here, not on the basis of his own experiments, but on those of von Jaksch, published the preceding year, he makes the "interesting discovery" that the relative amount of urea-nitrogen "seems" to depend upon the absolute amount of total nitrogen eliminated. Unfortunately the urea-determinations of von Jaksch which led Camerer to the above-mentioned deduction were soon afterwards shown by von Jaksch[2] to be much too low and altogether misleading, because they had been made according to the directions given in his textbook, instead of according to the original directions of Schöndorff, and the former (textbook) directions contain a misprint which proved fatal to the results. According to the textbook directions, he had in some cases found as little as 66 per cent of the total nitrogen as urea, but could obtain no such results when he had eliminated the mistake in his technique. Since Camerer's deductions concerning the relation between the absolute amount of total nitrogen and the per cent present as urea-nitrogen were drawn from admittedly erroneous experimental data, there is not much reason for believing that he still considers those deductions sound. As far as I am aware, he has published nothing on the subject since.

The results obtained from Dr. van Someren's urine showed, however, positively either that his metabolism was very different from the normal, or that the absolute amount of nitrogen in urine determines to a heretofore-unsuspected extent the per cent of it eliminated as urea. But feeding experiments with low nitrogen diets on other normal persons not previously accustomed to such diets have given in every case results similar to those shown by Dr. van Someren's urine. It may, therefore, be positively stated as a principle in the chemistry of metabolism that *the distribution of the nitrogen in urine among urea and the other nitrogenous constituents depends on the absolute amount of total nitrogen present.*

After having established by means of preliminary experiments with mixed low-nitrogen diets the correctness of this principle, further studies with uniform diets were undertaken. It did not seem improbable that such studies would yield considerable definite information concerning the conditions that determine the formation and elimination of each separate nitrogenous constituent. The investigations

[1] CAMERER: *Loc. cit.*, pp. 14–16. [2] JAKSCH: *Loc. cit.*

seemed, moreover, equally promising in connection with the sulphur of urine, for it was found that with respect to this element also there is in Table I an entirely different distribution among the three chief representatives (inorganic sulphates, ethereal sulphates, and neutral sulphur) from that previously observed in feeding experiments with the standard nitrogen-rich diet. (Compare Tables VII and VIII.) Since these observations have also been verified by the later experiments, these facts are also held to illustrate a principle in the chemistry of metabolism. *The distribution of the sulphur in urine among the three chief normal representatives — inorganic sulphates, ethereal sulphates, and "neutral sulphur" — depends on the absolute amount of total sulphur present.*

For a more detailed study of the laws or conditions governing the formation and elimination of the several more important urinary constituents the reader's attention is now called to Tables II–V. The four feeding experiments there recorded, all practically alike, are divided into three periods separated by double lines in the tables. During the first period, lasting three or four days, each person took the standard protein-rich food described in the preceding paper and which forms the basis of the results there recorded. At the end of this period it will be seen that each person eliminated exactly the same kind of urine as has already been abundantly illustrated in the first seven tables. A complete change of diet was then made. The effects of this second diet, containing only about 1 gm. of nitrogen as against 19 gm. in the first diet, are represented in the second period of the tables (the space between the two pairs of double lines). The second period lasted from seven to ten days. At the end of this time a return was made to the first protein-rich diet, but only for one or two days.

The diet used in the second period of these experiments is referred to in the tables as a "starch and cream diet." It is by no means an easy matter to provide a diet sufficiently nourishing for the ordinary normal man without including enough nitrogenous material to establish nitrogen-equilibrium, if the food is to be sufficiently palatable to enable a person to take it for more than two or three days. Yet it is only by means of such a diet that one can hope to bring the total amount of nitrogen in the urine down to the lowest possible level. The diet here selected is believed to be well adapted for the purpose, but the quantities used were somewhat too small to yield the most satisfactory results. The diet consisted of 400 gm. of pure starch and

TABLE II.

O. F. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. July.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
65.4 11	31 840	12.2	22.5 10.5	323.0 0.45	1.58 0.58	0.53 0.18	0.53	85.9	3.7	89.6	4.7	1.35	4.35
12	35 865	14.5	25.8 12.6	371.0 0.52	1.66 0.64	0.64 0.21	0.57	86.7	3.6	90.3	4.4	1.4	3.9
65.1 13	36 850	16.1	30.2 14.1	360.0 0.50	1.49 0.55	0.66 0.22	0.70	87.5	3.2	90.7	3.4	1.4	4.5
14	35 520	10.6	19.1 8.9	360.0 0.50	1.38 0.51	0.50 0.17	0.46	84.4	4.7	89.1	5.0	1.6	4.3
15	29 475	7.8	13.1 6.1	327.0 0.46	1.39 0.52	0.48 0.16	0.48	79.0	5.9	84.9	6.7	2.1	6.3
16	20 600	6.5	10.1 4.8	316.0 0.44	1.35 0.50	0.37 0.12	0.64	73.5	6.8	80.5	7.7	1.9	9.9
17	20 480	4.7	6.6 3.15	335.0 0.47	1.33 0.49	0.28 0.09	0.42	67.5	10.1	77.6	10.5	2.0	9.9
18	24 420	5.1	7.5 3.5	363.0 0.51	1.51 0.56	0.33 0.11	0.40	69.0	10.0	79.0	11.0	2.1	7.9
19	16 810	4.9	7.3 3.4	360.0 0.50	1.47 0.54	0.34 0.11	0.37	69.0	10.2	79.2	11.1	2.2	7.5
20	27 340	3.9	4.9 2.3	313.0 0.44	1.48 0.54	0.32 0.11	0.49	60.0	11.4	71.4	14.0	2.8	12.8
21	27 300	4.1	5.6 2.65	310.0 0.43	1.48 0.54	0.34 0.11	0.32	65.3	10.7	76.0	13.3	2.7	8.0
22	.. 460	4.2	5.7 2.7	352.0 0.49	1.60 0.60	0.32 0.11	0.43	61.8	11.5	73.3	14.0	2.6	10.1
23	27 315	3.8	4.9 2.3	368.0 0.52	1.57 0.58	0.35 0.12	0.25	61.2	13.6	74.8	15.4	3.1	6.7
62.8 24	22 1.00	9.8	15.7 7.3	545.0 0.76	1.60 0.60	0.55 0.18	0.91	74.9	7.8	82.7	6.1	1.9	9.3

TABLE II — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{N}{10}$.			Total phosphates as P_2O_5.	Chlorine gm.	Indican (Fehling's solution = 100).	
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
11	2.78	2.46	0.17	0.15	88.5	6.1	5.4	443	−25	468	2.9	5.4	60	
12	3.46	3.13	0.19	0.14	90.4	5.5	4.1	557	308	249	3.2	5.9	100	
13	3.33	3.03	0.18	0.10	91.2	5.4	3.4	867	370	497	4.1	6.0	50	
14	1.45	1.13	0.12	0.10	78.0	8.3	13.7	497	308	189	2.4	3.2	50	
15	0.99	0.80	0.08	0.11	80.8	8.1	11.1	371	154	217	1.8	2.6	trace.	
16	0.86	0.60	0.10	0.16	70.0	11.6	18.4	295	26	269	1.4	2.8	0	
17	0.83	0.62	0.08	0.13	74.7	9.6	15.7	235	55	180	1.1	2.6	0	
18	0.79	0.54	0.07	0.18	68.3	9.0	22.7	241	29	212	1.1	2.6	0	
19	0.75	0.53	0.06	0.16	70.7	8.0	21.3	259	17	242	1.2	3.8	0	
20	0.72	0.44	0.09	0.17	61.1	12.5	26.4	264	5	269	1.1	2.3	0	
21	0.79	0.51	0.11	0.17	64.6	12.6	22.8	96	5	91	1.0	1.7	0	
22	0.75	0.47	0.10	0.18	62.6	13.3	24.1	202	8	194	0.7	2.4	0	
23	0.74	0.42	0.09	0.23	56.7	12.2	31.1	222	−16	238	1.1	2.0	0	
24	1.90	1.53	0.19	0.18	80.5	10.0	9.5	286	−35	321	1.7	7.0	trace.	

TABLE III.

DR. H. B. H. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0—.	Total Nitrogen. N_2.	Urea. gm.	Ammonia. c c. $\frac{1}{10}$ NH_3	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. July.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
87.0	*10*		*24.6*	*242.0*	*1.63*	*0.55*							
11	1440	13.4	11.5	0.34	0.60	0.18	0.78	85.8	2.5	88.3	4.4	1.3	6.0
12	*26* 1160	13.9	*25.7* 12.0	*305.0* 0.43	*1.57* 0.58	*0.53* 0 18	0.71	86.6	3.1	89.7	4.2	1.3	4.8
86.7	*28*		*32.1*	*351.0*	*1.58*	*0.53*							
13	1170	16.8	14.7	0.49	0.58	0.18	0.85	87.5	3.0	90.5	3.6	1.05	4.85
14	*29* 630	10.6	*18.9* 8.9	*292.0* 0 41	*1.36* 0.50	..	..	83 5	4.0	87.5	4 7	..	..
15	*18* 775	7.5	*13.1* 6.1	*298.0* 0.42	*1.49* 0.55	*0.34* 0.11	0.32	81.2	5 5	86.7	7.3	1 5	4.5
16	*08* 1580	6.7	*10.6* 5.0	*316.0* 0.43	*1.49* 0.55	*0.29* 0.10	0.62	73.9	6.4	80.3	8.2	1.5	10.0
17	*10* 890	4.4	*6.4* 2.9	*302.0* 0.42	*1.43* 0.52	*0.32* 0.11	0.45	66.8	9.6	76.4	11.8	2.4	9.4
18	*06* 1880	5.3	*7.5* 3.5	*338.0* 0.47	*1.58* 0.58	*0.26* 0.09	0.63	69.0	9.0	78.0	11.6	1.7	8.0
19	*06* 1600	4.8	*6.5* 3.0	*365.0* 0.51	*1.61* 0.61	*0.28* 0 09	0.58	63.6	10.7	74.3	12.9	1.9	10.9
85.7	*21*		*4.7*	*299.0*	*1.60*	*0.26*							
20	385	3.6	2.2	0.42	0.60	0.09	0.27	61.7	11 3	73.0	17.2	2.5	7.3
21	*27* 745	9.1	*16.0* 7.4	*405.0* 0.56	*1.65* 0.63	*0.49* 0.16	0.35	78.8	6.2	85.0	6.9	1.7	[illegible]
22	*26* 975	13.7	*25.1* 11.7	*456.0* 0.64	*1.58* 0.58	*0.54* 0.18	0.60	83.7	4.7	88.4	4.2	1.3	6.1

TABLE III — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
11	2.81	2.44	0.17	0 20	86.8	6.1	7.1	461	225	231	2.6	6.2	40	Milk and egg diet.
12	3.40	2.80	0.26	0.34	82.4	7.6	10.0	626	204	422	3.4	6 3	120	
13	3.64	3.27	0.19	0.18	90.0	5.2	4.8	805	398	407	4.1	6.1	120	
14	1.47	1.13	0.15	0.19	77.0	10.2	12.8	504	174	330	2.4	2.5	20	Starch and cream diet.
15	1.07	0.67	0.11	0.29	62.6	10.3	27.1	419	204	215	2.2	1.9	0	
16	0.92	0.56	0.14	0.22	61.0	15.2	33.8	291	57	234	1.3	2.3	0	
17	0.71	0.40	0.10	0.21	56.3	14.1	29.6	249	82	167	1.1	2.3	0	
18	0.77	0.40	0.09	0.28	52.0	11.7	36.3	226	38	188	1.0	7.0	0	
19	0.83	0.42	0.10	0.31	50.6	12.0	37.4	205	..	..	0.85	4.8	0	
20	0.76	0.46	0.10	0.20	60.5	13.2	26.5	224	123	201	1.0	1.6	0	
21	2.59	2.30	0.12	0.17	88.8	4.6	6.8	375	107	268	1.6	3.6	+	Milk andegg diet.
22	3.12	2.68	0.22	0.22	85.9	7.0	7.1	534	320	214	2.6	5.0	10	

TABLE IV.

DR. E. S. A. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. June-July.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
56.3	*32*	11.1	*20.2*	*265.0*	*1.11*	*0.47*	0.74	85.0	3.3	88.3	3.6	1.4	6.7
28	780		9.4	0.37	0.40	0.16							
29	*24*	14.6	*26.9*	*377.0*	*1.05*	*0.44*	0.96	86.0	3.6	89.6	2.7	1.0	6.6
	1240		12.6	0.54	0.39	0.15							
30	*15*	15.8	*29.7*	*377.0*	*1.16*	*0.34*	0.88	87.7	3.3	91.0	2.7	0.7	5.6
	2300		13.9	0.54	0.43	0.11							
55.6	*17*	14.4	*26.0*	*394.0*	*1.12*	*0.33*	0.79	87.2	3.8	91.0	2.8	0.7	5.5
1	1860		12.5	0.55	0.40	0.11							
2	*13*	8.6	*14.4*	*390.0*	*1.17*	*0.22*	0.89	77.7	6.3	84.0	5.0	0.8	10.2
	1220		6.7	0.54	0.43	0.07							
3	*10*	5.3	*8.7*	*224.0*	*1.14*	*0.23*	0.39	77.3	6.0	83.3	8.0	1.4	7.3
	565		4.1	0.31	0.42	0.08							
4	*12*	5.3	*9.0*	*288.0*	*1.07*	*0.23*	0.19	79.7	7.6	87.3	7.6	1.4	3.7
	960		4.2	0.40	0.40	0.08							
5	*9*	4.4	*7.1*	*252.0*	*1.09*	*0.29*	0.29	74.2	8.0	82.2	9.2	2.1	6.5
	1075		3.3	0.35	0.41	0.10							
6	*17*	2.7	*3.4*	*243.0*	*1.17*	*0.23*	0.30	58.2	12.4	70.6	15.7	2.6	11.1
	505		1.6	0.34	0.43	0.08							
7	*15*	3.7	*5.3*	*121.0*	*1.21*	*0.43*	0.39	69.0	4.6	73.6	12.0	3.9	10.5
	670		2.5	0.17	0.44	0.14							
55.7	*17*	2.8	*3.6*	*202.0*	*1.13*	*0.25*	0.32	60.7	10.0	70.7	14.0	3.0	11.6
8	700		1.7	0.28	0.41	0.08							
9	*16*	7.1	*11.7*	*316.0*	*1.10*	*0.31*	0.61	77.7	6.2	83.9	6.1	1.4	8.6
	1490		5.5	0.44	0.43	0.10							
55.4	*22*	9.4	*17.0*	*330.0*	*1.22*	*0.34*	0.46	84.4	4.9	89.2	4.7	1.2	4.9
10	1100		7.9	0.46	0.44	0.11							

TABLE IV — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
28	2.55	2.21	0.18	0.16	86.7	7.1	6.2	593	246	247	3.3	5.1	150	Milk and egg diet.
29	3.02	2.56	0.26	0.20	84.7	8.6	6.7	719	401	318	4.1	6.7	180	
30	2.94	2.58	0.22	0.14	87.7	7.4	4.9	690	530	160	4.3	8.6	150	
1	3.01	2.66	0.19	0.16	88.3	6.0	5.7	707	298	409	4.1	7.4	80	
2	1.35	1.00	0.15	0.20	74.1	11.1	14.8	537	210	327	2.8	3.0	30	Starch and cream diet.
3	0.88	0.67	0.10	0.11	76.1	11.4	12.5	325	97	228	1.4	2.0	trace.	
4	0.65	0.46	0.07	0.12	70.8	10.8	18.4	303	65	238	1.3	1.8	trace.	
5	0.61	0.45	0.06	0.10	73.7	10.0	16.3	253	97	156	1.2	2.8	trace.	
6	0.51	0.32	0.07	0.12	62.7	13.7	23.6	217	−20	237	0.9	2.4	0	
7	0.85	0.68	0.07	0.10	80.0	8.2	11.8	107	−364	471	0.6	5.7	0	Potato diet.
8	0.64	0.42	0.07	0.15	65.6	10.9	23.5	207	−291	498	0.8	4.5	0	
9	1.63	1.39	0.12	0.12	85.2	7.4	7.4	345	−220	565	2.2	8.6	0	Milk and egg diet.
10	2.64	2.35	0.16	0.13	88.6	6.1	5.3	594	211	383	2.6	6.3	20	

TABLE V.

DR. AUG. H. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0 —.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. June-July.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
70.1	30	14.4	26.6	328.0	1.48	0.59	0.79	86.1	3.2	89.3	3.8	1.4	5.5
28	1065		12.4	0.46	0.55	0.20							
29	30	14.8	27.7	324.0	1.32	0.54	0.78	87.1	3.1	90.2	3.3	1.2	5.3
	980		12.9	0.45	0.49	0.18							
30	30	15.7	29.1	388.0	1.31	0.43	0.94	86.5	3.5	90.0	3.1	0.9	6.0
	1000		13.6	0.54	0.48	0.14							
70.1	29	16.8	31.4	385.0	1.43	0.49	0.86	87.5	3.2	90.8	3.1	1.0	5.1
1	1080		14.7	0.54	0 53	0.16							
2	29	9.9	18.0	242.0	1.29	..	..	84.5	3.4	87.9	4.3	..	..
	535		8.4	0.34	0.48								
3	30	7.6	12.6	227.0	1.32	0.42	0.74	78.1	4.2	82.3	6.4	1.6	9.7
	440		5.9	0.32	0.49	0.14							
4	25	5.9	9.7	242.0	1.28	0.37	0.51	76 0	5.7	81.7	8.0	1.6	8.7
	475		4.5	0.34	0.47	0.12							
5	28	5.4	8.6	237.0	1.37	0.30	0.47	73.1	6.4	79.5	9.3	2.4	8.8
	405		4.0	0.33	0.51	0.13							
6	26	3.5	4.3	239.0	1.31	0.30	0.54	57.9	9.4	67.3	13.6	3.7	15.4
	380		2.0	0 33	0.48	0.13							
7	14	4.2	6.0	123.0	1.44	0.48	0.48	67.4	4.0	71.8	12.7	4.0	11.5
	730		2.8	0.17	0.54	0.16							
70.7	24	3.5	4.5	197.0	1.37	0.41	0.52	59.3	7.8	67.1	14.1	4.0	14.8
8	585		2.1	0.27	0.50	0 14							
9	19	6.8	11.6	237.0	1.32	0.32	0.47	79.2	4.8	84.0	7.2	1.9	6.9
	1260		5 4	0 33	0.49	0.11							
10	19	10.9	19.1	258.0	1.43	0.43	0.95	81.7	3.3	85.0	4.9	1.3	8.8
	1375		8.9	0.36	0.53	0.14							

TABLE V — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{N}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
28	3.32	2.84	0.23	0.23	85.5	7.2	7.3	626	331	295	3.5	7.8	80	Milk and egg diet.
29	3.09	2.71	0.20	0.16	87.7	6.5	5.8	764	353	411	4.1	6.3	150	
30	3.27	2.82	0.22	0.23	86.2	6.5	7.3	828	464	364	4.4	6.0	150	
1	3.34	2.99	0.19	0.16	89.5	5.7	4.8	799	363	436	4.6	5.8	150	
2	1.45	1.11	0.15	0.19	76.6	10.3	13.1	543	265	278	2.8	2.3	50	Starch and cream diet.
3	1.05	0.79	0.11	0.15	75.2	10.5	14.3	409	178	231	1.9	1.9	trace.	
4	0.88	0.59	0.13	0.16	67.1	14.7	18.2	324	79	245	1.4	2.7	trace.	
5	..	0.68	0.07	..	..	..	..	334	36	298	1.5	2.7	trace.	
6	0.65	0.41	0.09	0.15	63.1	13.8	23.1	266	12	254	1.0	1.8	0	
7	0.88	0.65	0.10	0.13	73.8	11.4	14.8	117	−467	584	0.8	6.5	0	Potato diet.
8	0.77	0.48	0.08	0.21	62.3	10.4	27.3	243	−182	425	1.2	5.1	0	
9	1.43	1.13	0.11	0.19	79.0	7.7	13.3	312	−85	397	1.5	7.2	0	Milk and egg diet.
10	3.20	2.76	0.20	0.24	86.2	6.3	7.4	528	88	440	3.1	5.6	30	

300 c.c. of cream, containing 15 to 20 per cent fat. Pure arrow-root was selected for the purpose, not only because it is free from nitrogen, but because it is more palatable than ordinary potato or corn starch. The starch mixed with 1500 c.c. of water, and heated until a uniform paste was obtained, was then cooled to 70° C., and digested at this temperature for about half an hour with 2 gm. of active diastase. The ferment reaction was stopped at the proper point by heating for a few minutes to 90° C. The operation is best carried out by means of a so-called double boiler. In this way one can easily obtain a mixture exceedingly rich in starch which is not sweet, and which is liquid when hot, and semi-solid when cold. The consistency can be regulated at will by means of longer or shorter action of the ferment, but the ferment action must not be carried too far, or the product will acquire a rather disagreeable sweetish taste. Six grams of sodium chloride was the only seasoning used, and no tea, coffee, alcohol, or stimulants of any kind were allowed during the experiments.

This diet has now been tried on several different normal persons, as well as on patients, and I have as yet found only one who was not able to take it, and he succumbed the first day. No person taking the diet has felt less able than usual to do his daily work, nor has any one reported any particular craving for nitrogenous food, though the diet of course gets to seem less and less palatable, especially when served with such unnecessarily small quantities of cream as were here used. Instead of 300 c.c. of cream, it would have been better to use 600 c.c., as the total nitrogen in the food would still have been below that needed for nitrogen-equilibrium, and the increased caloric value of the food would then in all probability have reduced the urinary nitrogen still further.

In the general outline nothing new or original is claimed for the feeding experiments with this diet. The conditions are essentially similar to those prevailing in most attempts that have been made to discover the lowest possible level of nitrogen-equilibrium, as, for example, in those of Sivén,[1] and they resemble still more the similar experiments of Landergren,[2] both of whom have in fact succeeded in reducing the nitrogen-elimination to a lower level than was here attained. But the main purpose here aimed at was exactly the reverse of theirs. Their chief aim was to reduce the protein-metabolism to the lowest possible level, and incidentally they studied somewhat the

[1] Sivén: *Loc. cit.*

[2] Landergren: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 112.

composition of the urine. In these experiments the attempt was made to reduce the protein-metabolism to a minimum, but only in order to make more instructive the data obtained from a detailed study of the composition of urine.

Turning now to Tables II to V, and taking first a general glance on the one hand at the total nitrogen and the total sulphur columns, and on the other at the columns representing the percentage-distribution of the different nitrogenous constituents and sulphur compounds, it will be seen that the two principles stated above with reference to this distribution hold true. With the change from the nitrogen-rich to the nitrogen-poor diet there is a great reduction in the total amounts of nitrogen and sulphur in the urine, and accompanying this diminution we find a most striking transposition in their distribution among the different constituents. The same fact is further illustrated in Tables VI–X. With the reduction of the total nitrogen and total sulphur in the urine, we find a smaller per cent represented as urea and as inorganic sulphates, and a relative increase in the other representatives of these two elements.

Some more detailed questions must, however, now be raised, and the answers sought for in the tables. Is the changed percentage-composition accompanying a reduced protein-metabolism due only to a reduction in the absolute amounts of urea and inorganic sulphates formed and eliminated? Do the other constituents also diminish, but at a different rate, or do they remain fixed in absolute amounts, or can they even be absolutely greater when the protein-metabolism is reduced? The reader has undoubtedly already asked these questions, and observed that the answers must be different for the different constituents.

In a discussion of the relative and absolute variations in the elimination of the nitrogenous constituents produced by quantitative changes in the protein-metabolism, it would perhaps seem most suitable to begin with urea, as is the general custom in all metabolism experiments in which it has been determined. In this case, however, the discussion of the urea will require considerable reference to the other nitrogenous constituents, and it will therefore be taken up only after they have been considered.

Kreatinin. — The part played by the kreatinin as a factor in the relative distribution of the urinary nitrogen is perhaps the most interesting fact discovered in the course of these investigations. It has already been pointed out (p. 68), in connection with Tables VII

and I, that when the urea constituted 86–89 per cent of the total nitrogen in Table VII, the kreatinin represented only 3.5–4.5 per cent; while in Table I, when the urea had sunk to 62 per cent of the total nitrogen, the kreatinin-nitrogen had risen to 11 per cent. The importance of the kreatinin as a representative of the protein-metabolism, when the latter has been reduced to a minimum, is even more strikingly illustrated in Tables II–V. In Table II the kreatinin-nitrogen represents 3.4 per cent of the total on the last day of the protein-rich diet, and 15.4 per cent on the last day of the starch and cream diet. The corresponding days in Table III give the values 3.6 and 17.4 per cent respectively. And almost identical changes in the relative importance of the kreatinin-nitrogen are shown by the other tables.

Turning from the consideration of the per cent in terms of the total nitrogen to that of the absolute quantities of kreatinin eliminated, we find the remarkable fact that *the absolute quantity of kreatinin eliminated in the urine on a meat-free diet is a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated.* This appears to me to be another fixed principle in the chemistry of metabolism. In verification of it, the reader is invited to inspect all the tables in this, as well as in the preceding paper, representing sixteen different feeding experiments, and 163 twenty-four-hour quantities of urine. The statement will be found substantially correct; certain small variations do occur, but the constancy of the kreatinin, as compared with all other nitrogenous constituents, is certainly remarkable, especially in view of the fact that the muscles and most if not all other organs must at all times contain very considerable quantities of kreatinin. The variations that do occur are as independent of the volume of the urine as of the total nitrogen. To cite but one out of very many illustrations of this to be found in the tables, the reader is referred to Table IV. There we find that Dr. A. eliminated, on June 30, 2300 c.c. of urine containing 15.8 gm. total nitrogen, and on July 6 he eliminated only 505 c.c. of urine and 2.7 gm. total nitrogen. The amount of kreatinin is exactly the same on those two days.

If the kreatinin findings here reported are correct, and it would seem as though the experimental material furnished constitutes sufficient evidence as to this, we have in the kreatinin by far the most reliable index as to the amount of a certain kind of protein-metabolism occurring daily in any given individual.

While the amount of kreatinin eliminated with the urine is for each individual practically a constant quantity, independent of the total amount of nitrogen and of the volume of the urine eliminated, the amount may be very different for different persons. Thus we find the average kreatinin-elimination of Dr. H., weighing 87 kgm., to be 1.6 gm. (Table III); while in the next table (IV) we find that Dr. A., weighing about 56 kgm., eliminates on the average about 1.15 gm. kreatinin. The chief factor determining the amount of kreatinin eliminated appears to be the weight of the person. The proportion between the body-weight and the amount of kreatinin in the urine is, however, not very constant. Fat or corpulent persons yield less kreatinin per unit of body-weight than lean ones. This is illustrated in Tables II and V. There we find that F., who weighs 65 kgm., but is lean, eliminates about 1.6 gm. kreatinin (Table II); while Dr. H. (Table V), who weighs 70 kgm., but is rather short and corpulent, yields 1.4 gm. kreatinin. The analytical data, as far as yet obtained, indicate that moderately corpulent persons eliminate per twenty-four hours about 20 mg. kreatinin per kilo of body-weight, while lean persons yield about 25 mg. per kilo.

The above facts show that in metabolism studies of the sick it is not permissible to speak of abnormally high or low kreatinin in the urine, without taking into account, on the one hand the body-weight, and on the other hand the physical condition, *i. e.*, the adipose tissue present.[1] In view of the seemingly extremely important significance of the kreatinin in the metabolism of normal persons, it would appear highly desirable that the elimination of this substance in different diseases be more fully investigated. Also it should be worth while to investigate the effect of certain drugs on the kreatinin-elimination, particularly those which are known to affect the uric acid, as, for example, the salycilates. The effect of hard physical exercise on the kreatinin-elimination has already been the subject of considerable investigation. The results may still be considered doubtful, and further experiments are necessary to settle this question. Gregor,[2] the most recent investigator in this field, came to the conclusion that hard physical exercise does produce a decided increase in the kreatinin-elimination, particularly on the day following such exercise. The extraordinary variations in the normal daily kreatinin-elimina-

[1] See Macleod, J. J. R.: Journal of physiology, Proceedings of the Physiological Society, xxvi, p. vii, 1900.

[2] Gregor: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 98.

tion shown by Gregor's figures render them, however, decidedly doubtful, although I am inclined to believe that his main contention may be correct.

The fact that the kreatinin-elimination is normally an almost perfectly constant quantity in any given person is important in connection with metabolism experiments in hospitals, or whenever one must depend on someone else for the proper collection of twenty-four-hour quantities of urine. Any considerable loss of urine is promptly shown by the kreatinin-determination, which can be made in a few minutes. As an illustration of this, attention may be called to the omission in Table VIII of the urine corresponding to April 18. 600 c.c. of urine, containing 5.1 gm. nitrogen, 136 c.c. $\frac{n}{10}$ ammonia, and only 0.81 gm. kreatinin, were brought to the laboratory as the full twenty-four-hour quantity. The preceding six days showed, however, that the patient should certainly not yield less than 1 gm. kreatinin per day. An investigation of the ward was at once instituted, and it was found that the patient had been without supervision during a considerable part of the day.

Uric acid. —The preceding discussion of kreatinin has doubtless reminded the reader of the important papers of Sivén,[1] and of Burian and Schur[2] on uric acid. Burian and Schur devote some two hundred and fifty pages to a very able and interesting discussion of the part played by the "purin bodies" in metabolism. The most important point brought out by them, or at any rate the point most pertinent in connection with these investigations, is their contention that the uric acid eliminated by man on a purin-free diet is for each individual a constant quantity and entirely independent of the total amount of nitrogen eliminated. This contention becomes peculiarly strong in view of the fact that Sivén simultaneously and independently had drawn the same conclusion from a series of experiments which seem particularly well adapted to prove the point.

Since the two diets described above (pp. 64 and 82) conform to the conditions demanded by Burian and Schur with regard to their purin contents, the numerous uric acid determinations recorded in these tables should throw additional light on the validity of the conclusion reached by Sivén and by Burian and Schur. By inspection of Tables II–V, it will, however, be seen that the uric acid elimination is by no means as great on the starch and cream diet as on the milk and egg

[1] Sivén: Skandinavisches Archiv für Physiologie, 1901, xi, p. 123.

[2] Burian and Schur: Archiv für die gesammte Physiologie, 1900, lxxx, p. 241; 1901, lxxxvii, p. 239; 1903, xciv, p. 273.

diet. In Tables II and III it sinks on the former diet to only about one-half of what it is on the latter; in Table IV it sinks to about two-thirds, and in Table V to about four-fifths. That is, under the conditions of these experiments, the uric acid elimination does not show the characteristic quantitative constancy that we find in the case of the kreatinin. *When the total amount of protein-metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent of the uric acid nitrogen in terms of the total nitrogen is therefore much increased.*

The last brief statement represents, however, by no means all that there is to be said concerning the conditions governing the uric acid elimination. This has always been a complex problem, and as far as I can see, is so still.

In the first place it might be said that the results obtained by Burian and Schur do not necessarily conflict with those recorded in Tables II–V. These authors did not work with urines showing such wide variations in their total nitrogen-contents as is here the case, and they accordingly generally refrain from claiming that the "endogenous" uric acid is absolutely independent of the total amount of protein-metabolism. It might therefore well be the case that the conditions prevailing in Experiments II–V are simply outside the limits within which the rule of Burian and Schur is true. But the feeding experiment of Sivén represents even wider variations in the urinary nitrogen than are shown in these tables, yet his results fully confirm the findings of Burian and Schur. In order to get light as to the cause of these different results, it seems necessary to sift out the experimental material that really has a bearing on the subject.

Notwithstanding the abundance of highly interesting experimental material recorded in the papers of Burian and Schur, it seems to me that their position with reference to the "endogenous" uric acid is not particularly well fortified. One single feeding experiment, lasting twelve days, and including nine uric acid determinations, constitutes all the experimental data of their own which they have recorded as a basis for their contention that the "endogenous" uric acid is for each individual a constant quantity.[1] Their experiment was divided into three four-day periods, and represents a diminution of 6 gm. in the total urinary nitrogen, *i. e.*, from 14 gm. to 8 gm.

Sivén also offers but one series of experiments in proof of the

[1] Burian and Schur: *Loc. cit.*; lxxx, p. 290; xciv, p. 274.

contention that the uric acid in the urine of man is independent of the total amount of nitrogen eliminated. His is, however, an exceedingly effective experiment, and fully justifies the conclusion which he drew from it. The experiment lasted thirty-nine days, and the urinary nitrogen was reduced from 21.1 gm. to 2.8 gm., yet the uric acid elimination remained practically constant.

Since the experiments recorded in Tables II to V show an unmistakable reduction of the uric acid when the total amount of protein-metabolism has been reduced to the extent there accomplished, we must conclude that the endogenous uric acid elimination can be reduced, at least under certain conditions. Burian and Schur must not be represented as denying this. They take due notice, for example, of the fact shown by Ranke and by Hofmann [1] that the amount of uric acid eliminated in hunger is actually less than that eliminated on a food containing no protein at all, and notwithstanding the fact that in the former case more total nitrogen is eliminated. But Burian and Schur consider all such results abnormal, and the result of a greatly changed metabolism. I have no doubt that they will pass the same judgment on the results recorded here. This seems, however, to me to be decidedly an open question, especially in view of the fact that the uric acid is less and not greater in hunger than on a food containing no protein. The fact that the kreatinin *is* a constant quantity in these experiments seems to me to be of no little importance in this connection. It is not easy to understand how "Einschmelzung xanthinbasenhaltigen Materials (Muskel u. s. w.)" [2] could take place without noticeably affecting the kreatinin-elimination one way or another.

If the endogenous uric acid is to be considered as derived from the cell nucleins exclusively, it would indeed seem highly plausible that the quantity should tend to remain constant, even with very great variations in diet. Rigid proof that the endogenous uric acid elimination is for each individual a constant quantity would be strong evidence in favor of such a theory. Burian and Schur support the view that the endogenous uric acid is derived from the cell nucleins, but they contend that in man about one-half of the uric acid so derived is destroyed inside the organism, and that only the other half is eliminated. With the introduction of this important modification of the nuclein theory, there is no longer any reason why the uric acid

[1] Burian and Schur: *Loc. cit.*; lxxxiv, p. 275.
[2] Burian and Schur: *Loc. cit.*; lxxx, p. 275.

elimination should not be a decidedly variable factor which might well be susceptible to change under the influence of many different changes in the conditions, among others, changes in diet. In any event it seems clear that the two experiments of Sivén and of Burian and Schur are not sufficient to prove the point under discussion, since these experiments have given different results.

Looking only at the analytical data of my experiments I am quite as much impressed by the variations in the uric acid elimination as by the absence of variations. The elimination tends in some cases to be relatively constant, but this tendency is broken into by a number of peculiar changes for which I have as yet found no explanation. To illustrate this, let us run over the different tables.

In Tables II and III the uric acid elimination sinks on the starch and cream diet to about one-half of what it is on the "purin-free" milk and egg diet. Tables IV and V show a similar but less pronounced diminution. One particularly interesting fact is to be noted in connection with the latter two tables. These two experiments were, as will be seen from the dates, carried on simultaneously. But on July 7 both subjects consumed about 2 kgm. of potatoes instead of the arrow-root starch. One of the subjects being somewhat tired of the pure starch, this change was tried for one day.[1] It was thought that this would not materially change the analytical results, except with reference to the acidity columns and the ammonia. But the uric acid elimination was also distinctly affected, particularly in the case of Dr. A. (Table IV). The uric acid elimination in this case was almost doubled on the potato day. The rise in the case of Dr. H. is less pronounced but still noticeable. The smaller rise in the latter case is probably in some way connected with the interesting fact that the preceding fall in the uric acid output under the influence of the starch and cream diet was very much smaller than in Tables II–IV. In view of the marked change in the uric acid elimination which accompanied the substitution of potatoes for pure starch, it is interesting and suggestive to note that the diet used in the experiments of Burian and Schur and Sivén contained respectively 500 gm. and 600 gm. potatoes. Burian and Schur[2] assert that

[1] It may be remarked here that the consumption of about 400 gm. of starch in the form of potatoes, even for one day, proved a much more difficult task than the taking of the arrow-root, and the next day both Dr. A. and Dr. H. expressed a decided preference for the pure starch.

[2] Burian and Schur: Archiv für die gesammte Physiologie, 1900, lxxx, p. 269.

in ordinary food, except such as contain "purin," *i. e.*, chiefly meat products, there are no other constituents which affect the "purin" elimination. In view of the above-mentioned result given by potatoes, when compared with the results given by pure starch, the experiments of Burian and Schur do not seem very convincing, although I am as yet not prepared to assert that potatoes do affect the uric acid elimination.[1]

Tables VI and VII represent no uniform definite diet, but only two preliminary experiments made to verify the findings in Dr. van Someren's urine (Table I). The diet was mixed, but so selected as to reduce the nitrogen, *i. e.*, no meat products were eaten, and only limited quantities of bread (white), milk, or eggs.

The uric acid figures here obtained can scarcely be said to disprove the contention that the endogenous uric acid is for each individual a constant quantity. In fact were it not for the experiments recorded in Tables II–V, and for some later ones, these two mixed diet experiments might be quoted rather in support of that contention, for the reduction in the uric acid elimination is indeed very small, notwithstanding that the total nitrogen elimination has been reduced by 7 gm. and 10 gm., respectively, *i. e.*, from 10.6 to 3.8 and from 15.9 to 6.3.

Let it be understood that I am in no sense denying that a certain part of the uric acid eliminated in human urine is "endogenous" in the sense that it is a product of tissue-metabolism, as distinguished from the products formed in the breaking down of non-living protein in the animal organism. In fact I could not come to any other conclusion from the uric acid values recorded in all these experiments. They all indicate that the more the total protein-metabolism is reduced, the more prominent in per cent of the total urinary nitrogen becomes the uric-acid-nitrogen, and the most probable explanation of such a relative increase in the uric-acid-nitrogen is, in my opinion, that at least a certain part of the uric acid represents the breaking down of living protoplasm which must be supposed to be essential to the continuation of life. We are, however, here considering whether *all* uric acid eliminated on a purin-free diet must be of such origin, and with reference to this question my experiments answer in the negative.

If the view advanced by Burian and Schur were correct, we should

[1] The uric acid determinations on July 7, in Tables IV and V, were repeated and found correct.

expect a fairly sharp line dividing their endogenous uric acid from the uric acid produced under the influence of food. They believe that they have found such a line of division. I have not been able to find it.

Even in Tables VI and VII it seems to me that a better case can be made for the negative side of the question. If we compare the three consecutive days having the highest total nitrogen in the urine, with the three days showing the lowest values, we find the following figures:

TABLE VI.

Feb. 1–3.	Total nitrogen, 29.8 gm.	Uric acid, 1.63 gm.	Kreatinin, 5.0 gm.
Feb. 11–13.	" " 13.4 gm.	" " 1.26 gm.	" 4.76 gm.

TABLE VII.

April 8–10.	Total nitrogen, 46.4 gm.	Uric acid, 2.3 gm.	Kreatinin, 5.5 gm.
April 31–June 2.	" " 20.1 gm.	" " 1.7 gm.	" 5.5 gm.

The comparison leads to the same conclusion as that stated on p. 87, namely, that a pronounced reduction in the urinary nitrogen is accompanied by an increase in the per cent of uric-acid-nitrogen, but by a diminution of the absolute quantity of uric acid.

Table VIII represents a feeding experiment with a patient, which is essentially similar to those recorded in Tables II–V. The uric acid elimination is here remarkably irregular, being at times as high on the starch and cream diet as on the milk and egg diet. A comparison of the last eight days with the first four days recorded will, however, show an unmistakable reduction. The result is 1.31 gm. as against 1.09 gm. (2.18 gm. for eight days). A noteworthy fact about this series of urines is that both the kreatinin and the uric acid are on the average noticeably less than the corresponding values ordinarily obtained from normal persons. This is especially true with regard to the kreatinin.

Table IX bears out fully all that has been said with reference to uric acid. On the starch and cream diet the uric acid is reduced to one-half, or less, of the amount eliminated on the milk and egg diet. A particularly striking comparison is furnished by the last day from each series, — 0.18 gm. uric acid on the former, as against 0.45 gm. on the latter. Yet the subject of the experiment did not even lose in weight on the starch diet.

Table X supports the general conclusion with reference to the uric acid. This series is, however, rather more interesting as a study

of metabolism in general paralysis. On June 13, the patient became suddenly almost unmanageable, and on that day there is to be noted a marked increase in the kreatinin, and a still more marked increase in the uric acid elimination.

These nine different feeding experiments all show that the uric acid elimination is reduced when the total nitrogen-elimination is very much diminished. The reduction is, however, irregular and different for different persons. The metabolic processes that determine the uric acid excretion may therefore be said to be in a relatively unstable equilibrium.

Ammonia. — With regard to ammonia as a product of metabolism, the following statement may be formulated as representing the results given by the tables: *With pronounced diminution in the protein-metabolism (as shown by the total nitrogen in the urine), there is usually, but not always, and therefore not necessarily, a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia-nitrogen, provided that the food is not such as to yield an alkaline ash.*

The conditions governing the ammonia-elimination in human urine produce, therefore, results very similar to those which we have already noted in connection with uric acid, though the determining factors themselves are probably very different. In the study of ammonia as a product of metabolism, it must be remembered that this substance is a base, and its formation in the animal organism is therefore probably quantitatively determined by the necessity of forming salts. Since we know that the human organism is capable of converting the ammonium salts of several organic acids into urea, we must conclude that the amount of ammonia appearing in the urine is determined by the character of the acids produced in the metabolism, *i. e.*, they cannot very well be acids the ammonium salts of which can be converted into urea and carbon dioxide. Such acids are, of course, primarily those derived from the sulphur and the phosphoric radicles of the decomposed protein-substances. But from certain forms of abnormal metabolism, and from the enormous increase in ammonia produced by the excessive consumption of fat,[1] we know that the formation of organic acids also may be equally effective in leading to an increased production of ammonia.

[1] LANDERGREN: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 125; JOSLIN: Journal of medical research, 1904, xii, p. 442.

The peculiar feature of the ammonia-elimination, as illustrated in the tables, is the pronounced individual differences shown under the influence of the starch and cream diet. These differences I am not able to explain. There are no corresponding differences to be found in the sulphate or phosphate elimination, nor do the organic acids in the urines show any corresponding variations. Table II, showing about 350 c.c. tenth normal ammonia per day on the starch diet, and Table IV, averaging a full 100 c.c. less, show the same amounts of organic acids, *i. e.*, about 225 c.c.; nor is the total acidity in the two appreciably different. But if the larger quantities of ammonia are not due to increased quantities of acids formed, then the excess of ammonia in one case above that in the other must be due to differences in the amounts of fixed bases eliminated by the two individuals. This much seems tolerably clear, but before attempting any further explanation, more analytical data must be obtained.

Undetermined nitrogen. — Nearly all attempts made in recent years to determine the relative distribution of the total urinary nitrogen have been based on the use of phosphotungstic acid as a precipitant. This reagent is supposed to precipitate, quantitatively, all the nitrogenous products except urea and traces of monoamido acids, and these are determined in the filtrate.

Total nitrogen, ammonia, and uric acid are determined separately, and by a series of additions and subtractions it is held that reliable figures for the so-called diamido acids are obtained. I have never found phosphotungstic acid a very satisfactory reagent for quantitative urine analysis,[1] and it must be admitted that the extensive investigations pursued during the past ten years, in accordance with the scheme outlined above, have not materially advanced our knowledge of the composition of human urine.[2]

By inspection of the columns giving the absolute amounts of nitrogen left undetermined in the present investigation, it will be seen that the direct determination of the four most important constituents, urea, kreatinin, ammonia, and uric acid, makes a much more satisfactory system of analysis than the elaborate indirect system based on

[1] Folin: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 509. See also Mörner: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 330, and especially the results of von Jaksch: Zeitschrift für physiologische Chemie, 1903, xl, p. 123.

[2] It was on the basis of results obtained by this system of analysis that von Jaksch came to the conclusion that the amount of urea present can be calculated directly from the total nitrogen.

the phosphotungistic acid precipitation. With but two exceptions (Tables VIII and IX), we find that the undetermined nitrogen is always less than 1 gm., even when the total urinary nitrogen is as high as 18 gm. or 19 gm. A detailed study of the different constituents that go to make up this nitrogenous residue would be very difficult in connection with such a comprehensive investigation as was here carried out. The only additional determination that could be considered practicable, would be that of the xanthin bases, and even their determination is far from satisfactory, unless a considerable fraction of the daily twenty-four-hour quantity of urine can be used.

From the variations of the undetermined nitrogen under the conditions prevailing in these feeding experiments, it seems clear that it must contain substances which, like kreatinin, or like ammonia and uric acid, increase in importance as the total amount of protein-metabolism is reduced; *i. e., the absolute quantity of undetermined nitrogen decreases under the influence of the starch and cream diet, but in per cent of the total nitrogen there is always an increase.* When the total urinary nitrogen has been reduced from 15 gm. to 3 or 4 gm., the undetermined nitrogen has decreased from 0.8 gm. or 0.9 gm. to 0.3–0.5 gm. Whether or no this relative increase in the undetermined nitrogen is due only to the xanthin bases must be left for future experiments to determine. It seems probable, however, that other substances showing a similar variation are present in this residue.

Urea. — On the preceding pages it has been shown that kreatinin, ammonia, uric acid, and the undetermined nitrogenous residue all become relatively more and more important as the total nitrogen in the urine decreases. We will now consider *urea, the only nitrogenous substance which suffers a relative as well as an absolute diminution with a diminution in the total protein-metabolism.* Urea, always considered the chief nitrogenous substance, and supposed to represent almost 90 per cent of the total nitrogen in normal human urine, as well as in nearly all pathological urines, has here been reduced in ten different cases to about 60 per cent.

It seems rather remarkable that this interesting behavior on the part of urea was not discovered long ago, because urines representing greatly reduced protein-metabolism have been studied by many different investigators. As far as I have been able to determine the reason seems to be purely a matter of analytical technique. This is clearly the case, for example, in the experiments of Landergren [1] made

[1] Landergren: *Loc. cit.*, p. 112.

only last year. The conditions of his experiments were almost identical with those here prevailing, yet when the urinary nitrogen had been reduced to 4 gm., the urea-determination made according to the Mörner-Sjöquist method, gave 84.8 per cent of the total nitrogen as urea. But the subject under investigation weighed 71 kgm., and the urine must therefore have contained about 1.6 gm. kreatinin or about 15 per cent of the total nitrogen. His own determination gave the ammonia as 5.6 per cent and the uric acid as 3.2 per cent. The undetermined nitrogen must have represented not less than 10 per cent. The method used in these experiments for the determination of urea would therefore almost certainly not have shown over 68 or 69 per cent of the total nitrogen in the above-mentioned urine as urea.

Had the diet used in Landergren's experiments not contained potatoes (200 gm.), the ammonia-nitrogen would have been increased by about 5 per cent, and the urea-nitrogen would then have represented at the most 65 per cent of the total nitrogen.

The reason why the remarkable variability of the urea-nitrogen, as per cent of the total nitrogen, was not discovered before, is therefore, I think, chiefly due to the fact that the earlier methods of determination gave erroneous results.

An interesting question to be considered here is, does 60 per cent represent about the lowest limit to which the urea-nitrogen can be reduced, or should it be possible to reduce it very much further? The answer to this question depends, it seems to me, wholly on the answer to another question, namely, whether it is possible to reduce the protein-metabolism to a still lower level? The results recorded in this paper show unmistakably that with every decided reduction in the total amount of urinary nitrogen the per cent of that nitrogen in the form of urea becomes less and less. If the total nitrogen could be reduced still further, there is every reason to believe that the per cent of urea-nitrogen should not only continue to diminish, but this diminution should become more and more rapid. To illustrate: If the last day on the starch and cream diet in Table II had given 1 gm. less of total nitrogen, there is no reason for supposing that this diminution should not have fallen almost if not wholly on the urea-nitrogen. We should then have had 2.8 gm. total nitrogen, and 1.3 gm. urea-nitrogen, *i. e.*, less than one-half of the nitrogen would have

existed as urea. In Sivén's experiments[1] the total nitrogen was reduced to this figure, and his subject weighed almost exactly the same as the subject represented by Table II. Another reduction of 1 gm. in the total nitrogen would under some conditions diminish the urea-nitrogen to less than 16 per cent of the total.

Is there any reason for believing that the urinary nitrogen can be reduced to this extent? This can, of course, only be decided by actually accomplishing it.

The analytical results recorded in this paper seem to me to indicate clearly the lowest possible limits that we can hope to attain for the nitrogen-elimination in man. It is conceivable that so long as any urea is eliminated it may still be possible to reduce the total nitrogen-elimination; but when the urea is absent, then the lowest *possible* limit has been reached. Is it *probable* that any such peculiar state of protein-metabolism can be produced in feeding experiments with normal men? I am not in position to express an opinion on this question. It depends on whether all the urea eliminated is of the same origin, or whether a certain part of it is produced in the same metabolic processes that give rise to the kreatinin.

Of great interest in this connection is the analytical result which I obtained a short time ago from a patient of this hospital. The patient had for several weeks taken extremely little food, and for five days preceding the collection of the urine he had taken no food of any kind, and in addition had abstained from drinking any water. 125 c.c. of urine was obtained, and this contained .85 gm. of nitrogen. Two closely agreeing duplicate urea-determinations were made. They showed that in this urine only 14.7 per cent of the total nitrogen was there as urea. 40 per cent of the nitrogen was, however, present as ammonia.

A still more interesting urine is one analyzed by Mörner[2] last year. This urine contained only 4.4 per cent of the total nitrogen as urea, with 26.7 per cent of nitrogen present as ammonia.

Of course both these urines were pathological, and for that reason cannot be used as conclusive evidence; but they suggest the possibility indicated above of reducing the urea in the urine of normal men to a much greater extent than has yet been accomplished.

[1] Sivén: *Loc. cit.*

[2] Mörner, K. A. H.: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 314.

III. The Distribution of the Sulphur of Urine among Inorganic Sulphates, Ethereal Sulphates, and "Neutral" Sulphur.

We have seen from the preceding tables that the relative distribution of the urinary nitrogen and sulphur among the different constituents depends upon the total amounts present, and we have discussed the nitrogenous constituents somewhat in detail. The sulphur constituents must now be considered.

The sulphur in urine is frequently used as a measure of the total amount of protein katabolism, and not infrequently we find that only the sulphates (*i.e.*, the mineral and ethereal sulphates) have been determined because the so-called neutral sulphur is supposed to represent but a small per cent of the total. But it is also recognized that the sulphur-elimination is much less accurate than the nitrogen as a measure of protein katabolism because of the well-known fact that different albuminous substances contain very different percentages of sulphur.

Aside from such general studies of the sulphur in metabolism, many special investigations have been pursued since Baumann made the important discovery that the sulphur in urine is not all in the form of inorganic sulphates. Baumann's own investigations were very extensive and resulted in the definite identification of a number of different conjugated or ethereal sulphates occurring in normal urine, and he also showed that they are all derived from aromatic products formed in the intestines by the action of bacteria on the protein of the food. The ethereal sulphates in general, and the indoxyl sulphate (indican) in particular, are therefore supposed to indicate the degree of intestinal putrefaction, the only subject of controversy being whether the per cent of ethereal sulphate or the absolute amount is to be considered the more important factor. More recently Blumenthal believed that the indican is a product of tissue-metabolism; but this view Ellinger[1] showed to be erroneous, so that the problem is at present supposed to be more or less definitely settled. The origin of the "neutral" sulphur, on the other hand, is still not understood, although this sulphur is supposed to be more or less directly derived from the bile acids.

As a part of the normal metabolism, the sulphur derivatives are

[1] Ellinger: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 44.

therefore at present believed to offer comparatively few and unimportant problems for further investigations.

But the analytical results obtained in the course of these metabolism experiments show that in the sulphur, as in the nitrogen-metabolism, there are certain regularities in the distribution of the waste-products.

In Tables II–V it is to be observed that as the total urinary sulphur is reduced, the per cent represented by the inorganic sulphates sinks from about 90 per cent to less than 60 per cent. The similarity of the inorganic sulphate elimination to that of the urea is too striking to be overlooked. But the reduction in per cent of inorganic sulphur must, of course, be accompanied by an increase of the other forms of sulphur, and by inspecting the tables it will be seen that both the ethereal sulphates and the neutral sulphur are increased in percentage of the total, that of the former being more than doubled, and that of the latter increasing four or five fold. Turning from the percentage figures to those giving the absolute quantities, we find that the ethereal sulphates diminish as the total amount of sulphur diminishes, but to a much smaller extent, and that the neutral sulphur is not visibly affected by the diminution of the total. In other words, the ethereal sulphate elimination is analogous to that of ammonia, uric acid, or undetermined nitrogen, and the elimination of neutral sulphur resembles that of the kreatinin.

The total sulphur-elimination, it will be seen, is, however, not nearly so constant as that of the kreatinin. I am inclined to ascribe this in a considerable extent to a lack of accuracy in the technique, because I have reason to believe that accurate sulphate determinations in urine are beset with greater sources of error than is generally recognized. In looking over a number of neutral sulphur-determinations recorded in the literature, I find even greater variations, variations so great, in fact, that they must certainly be due to analytical errors.[1]

These remarkable results can, it seems to me, be interpreted only on the basis of general processes of metabolism and not on the basis of intestinal putrefaction.

It will be noted that under the influence of the starch and cream diet the indican invariably disappears altogether from the urine, while the absolute quantity of ethereal sulphates is reduced only to

[1] See, for example, those of SIVÉN: Skandinavisches Archiv für Physiologie, 1901, xi, p. 325.

about one-half of the amount eliminated on the nitrogen-rich diet. Moreover, under the influence of the latter diet, the indican and the ethereal sulphates do not vary together. In Table IX, for example, we find the indican less than "50," with the ethereal sulphates amounting to 0.32 gm., while in Table V the indican is "150" and the ethereal sulphates amount to only 0.22 gm. Although the indican undoubtedly exists as an ethereal sulphate in urine, the experiments here recorded clearly speak against the view that the ethereal sulphates are all due to conditions identical or similar to those which give rise to the indoxyl sulphate.

On the basis of these analytical data the following conclusions seem warranted: (1) The urinary indican is not to any extent a product of the general protein-metabolism, is therefore probably, as is generally supposed, a product of intestinal putrefaction, and may consequently be assumed to indicate approximately the degree of putrefaction in the intestinal tract. (2) The ethereal sulphates can only in part be due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which the putrefaction is taking place in the intestines. (3) The ethereal sulphates, on the contrary, represent a form of sulphur-metabolism which becomes more prominent when the food contains little or no protein. (4) The neutral sulphur is not at all due to processes identical or similar to those which give rise to indican. (5) The neutral sulphur represents products which in the main are independent of the total amount of sulphur eliminated or of protein katabolized.

If the deductions here drawn are correct, if the inorganic sulphates, like urea, represent products that are chiefly involved in quantitative changes of the total sulphur and total nitrogen eliminated, and if, on the other hand, the ethereal and still more the neutral sulphur have an origin similar to that of uric acid and kreatinin, then we must expect that the amount of ethereal and neutral sulphur eliminated is more or less dependent on the kind of food taken. We know that meat products, in contradistinction to most other forms of protein, contain precursors of uric acid and kreatinin, and that on meat diets the elimination of these substances is much increased. Are the ethereal sulphates and neutral sulphur also greater on meat diets than on diets rich in protein but containing no meat? I have not been able to find any literature bearing directly on this important point. Neutral sulphur-determinations have been made frequently

enough, and the effects of different diets have been investigated. But the different percentages obtained can clearly not be interpreted as being due to the diets, so long as the effects of mere quantitative changes in the total sulphur were not recognized. The relative increase of the neutral sulphur on a bread diet, for example (Heffter), is thus to be explained, and does not at all warrant the conclusion that the sulphur of cereal proteins gives rise to different waste products in the metabolism. Experiments intended to show whether meat extracts contain sulphur constituents analogous to the nitrogenous purin bodies and kreatin are in progress.

IV. Water and Chlorides.

Water. — The volume of urine eliminated depends directly upon the amount of water consumed. This self-evident fact needs no discussion. But the variations in the volume of urine which cannot be traced to changes in the intake of water are of considerable interest. Such changes are at times exceedingly pronounced in the same individual, and different persons will, on the same diet, habitually eliminate very different amounts of water with the urine. Changes of this kind cannot be accounted for by changes in weight, and do not correspond to changes in the atmospheric conditions.

For example, on comparing the first periods of Tables II, III, and X, we find that on exactly the same amount of water consumed the volumes of urine eliminated during the three days are 2555 c.c., 3770 c.c., and 5500 c.c., respectively; yet the loss of body-weight is in each case about 300 gm., and the first two cases represent the same dates, thereby excluding the influence of the atmospheric conditions. Similarly in Table IV (first period) we find the volume of urine varying from 780 c.c. to 2300 c.c. without change of diet and with but a slight loss of body-weight. And in Table III (second period) we find the volume of urine varies from 385 c.c. to 1880 c.c. I have observed that the greatest volumes of urine frequently occur on days when the body gains in weight.

But changes in body-weight occurring in the course of feeding experiments with normal persons are unquestionably largely due to gains or losses of water. That this is so is shown in the second periods of the feeding experiments Tables II to X. The starch and cream diet contained not over 2200 calories; any extensive retention of fat is therefore practically excluded. Yet on this diet, involving

the loss of considerable quantities of nitrogen, we find frequently no loss of body-weight. In Table IX, for example, we find a loss of about 40 gm. of nitrogen with the urine alone, in the course of thirteen days, yet the body-weight remained perfectly constant. In Table X, 33 gm. of nitrogen were lost with the urine during seven days, but the body-weight increased 600 gm. If this nitrogen should be calculated in terms of muscle substance, it would amount to about 1 kgm., and this person would therefore have had to retain more than 1600 gm. of fat to explain the gain in weight. Positive proof that this person could not have retained this quantity of fat is obtained from the caloric value of the food compared with that of the fat. 1600 gm. of fat contain about 15,000 calories, while the entire food supply furnished during the seven days amounted to about 15,500 calories. In short, gain or loss of water is, within wide limits, an independent factor which may or may not coincide with the loss of nitrogen.[1] The volume of urine eliminated by normal persons is, therefore, largely a personal peculiarity, and is probably to a great extent inversely in proportion to the amount given off through the pores of the skin. The latter, the insensible perspiration, must therefore also not only be highly variable under different atmospheric conditions, as we know is the case, but it must normally be quite different for different individuals.

Chlorides. — The chlorine determinations made in connection with these feeding experiments have not yielded anything particularly new. It has long been known that the chloride-elimination varies chiefly with the volume of the urine, provided, of course, that the chlorine intake is constant. This conception is correct in so far as it is applied to any one individual, *i. e.*, on a uniform diet a person's chlorine-elimination varies to a considerable extent with the volume of the urine. This rule, however, does not hold for urines from different persons. For example, in Tables II, III, and X (first period) we found the total volume of three days' urine 2555 c.c., 3770 c.c., and 5500 c.c.; the total chlorine eliminated is, however, very nearly the same in all three, namely, 17.3 gm., and 18.6 gm. and 18.7 gm.,

[1] That muscle-preparations can, under different conditions, retain very different amounts of water, was shown several years ago by J. Loeb: Archiv für die gesammte Physiologie, 1894, lvi, p. 1. It is, therefore, not at all strange that the human organism should show a similar behavior, but it is important to know that a considerable loss of body-weight, as well as of nitrogen, can occur without signifying loss of muscle-substance or an impairment of vigor.

respectively. This is only what we must expect in view of the fact that the volume of urine corresponding to a constant intake of liquid is different for different persons.

V. The Relation of Phosphates and Organic Acids to the Acidity of Urine.

In the course of my metabolism experiments and acidity determinations I have arrived at a new point of view concerning the condition of the urinary phosphoric acid and the nature of the acidity of human urine. The accepted view concerning the subject may be briefly stated as follows: The normal elimination of acids and bases with the urine gives a mixture having an acid reaction, but on account of the tribasic character of phosphoric acid, and on account of the variable ammonia-production there is never any free acid present. The average normal result is an acidity equal to and due to a certain part (about 60 per cent) of the phosphoric acid present in the form of diacid-phosphate. Normal variations in the acidity are represented by variations in the amounts, absolute and relative, of the diacid-phosphates. Monoacid-phosphate is a slightly alkaline salt, diacid-phosphate is a rather weak acid, and their constant presence together constitutes an admirable arrangement for preserving the acidity in the form of the comparatively weak and harmless acid-phosphate.

The numerous attempts that have been made to procure a suitable and practical method for determining the degree of acidity have, accordingly, chiefly been directed toward a study of the acid-phosphate mixtures in the presence of the disturbing alkaline earths (calcium and magnesium). The most interesting of such attempts, in connection with the present discussion, are those of Freund[1] and Lieblein.[2] They determine directly the total phosphoric acid, then precipitate the monoacid-phosphate by the addition of barium chloride, and, by a second phosphoric-acid determination in the filtrate, get figures from which they calculate the acidity. The interesting part of this procedure is that it would seem to constitute valid proof that the phosphates in urine always do exist as a mixture of the mono- and diacid salts,— a view which, moreover, as far as I know, has never been questioned.

I believe, however, that I can now prove not only that the above method of Freund and Lieblein does not even approximately indicate

[1] Freund: Centralblatt für die medicinischen Wissenschaften, 1892, p. 689.

[2] Lieblein: Zeitschrift für physiologische Chemie, 1894, xx, p. 52.

the acidity of urine, but that the results obtained by the procedure of adding neutral barium-chloride to urine have practically no connection whatever with the acidity, and further that the phosphate precipitate obtained on adding barium chloride to urine does not at all prove that a certain part of the phosphates is in the dibasic form.

The principle of the method is of course approximately correct. When a soluble calcium or barium salt is added to a dibasic phosphate solution the insoluble di-calcium or di-barium phosphate is precipitated, and the presence of monobasic phosphate does not materially interfere with the reaction. The principle is correct, but the procedure as applied to urine is nevertheless grossly erroneous. The chlorides of calcium and barium show a similar behavior toward dibasic phosphate solutions; but in the study of the urinary phosphates, barium chloride is always specified as the reagent to be used. This choice seems strange. One would think that calcium chloride would be much more suitable, because the barium salt gives also a very abundant sulphate precipitate which so hides the presence of precipitated phosphate that mere inspection does not show whether or not any has been formed, and one must rely exclusively on the subsequent phosphoric-acid determination in the filtrate. The reason for this peculiar preference for barium is, however, not very difficult to find. *Barium chloride does, calcium chloride does not, give a phosphate precipitate with normal, clear, acid urines.* Why this important difference has been passed over in silence by the numerous investigators who must have observed it in connection with their studies of the acidity and the phosphates of urine seems almost inexplicable.

The idea that dibasic as well as monobasic phosphates are present in normal urine is so firmly fixed that the failure of calcium chloride as a precipitant probably has seemed to each observer accidental, and due to the presence of some disturbing factor that he did not care to search for. As a matter of fact, I myself failed to quite comprehend the full significance of my own analytical results in my paper on the acidity of urine because I could not doubt the correctness of the seemingly unquestionable existence of dibasic phosphates in urine. But having once fairly questioned the correctness of this view, the whole problem of the behavior of the urinary phosphates and their relation to the acidity appears in a new and seemingly clear light.

Calcium chloride does not give a precipitate with clear acid (litmus) urines, because the total acidity in such urines is equal to or greater than the acidity of the total phosphoric acid present, ex-

pressed as diacid salts, and therefore also there is no dibasic phosphate there. *The results obtained with barium chloride in such urines represent nothing more or less than errors due to the presence of sulphates.* Barium-sulphate precipitates have long been known to carry down all kinds of impurities, and I find that this is particularly true with reference to barium phosphate. If barium chloride is added to a pure standard monopotassium phosphate solution, no precipitate is formed; but if some ammonium sulphate is first dissolved in the solution, the barium sulphate formed on adding the barium chloride can at once be removed by filtration (in contradistinction to pure barium sulphate), and 30 to 40 per cent of the phosphoric acid is missing in the filtrate. This is evidently the reason why the Freund-Lieblein procedure always shows the urinary phosphoric acid to be present as a mixture of monoacid dibasic phosphates.

Soluble phosphates do, as a matter of fact, exhibit a very strong tendency to form precipitates with calcium as well as with barium, even when the solution contains no dibasic phosphate. For example, if to a pure monopotassium-phosphate solution is added first sodic acetate, and then calcium chloride, a precipitate is at once formed, and this precipitate dissolves only to a very small extent on adding acetic acid. If the acetate and acetic acid are added first, no precipitate may be formed when the calcium chloride is added, but it will appear when the solution is heated. This phenomenon is occasionally observed when the heat test is made for albumin in urine, only here the amounts of calcium and of organic salts present are small, and the phosphate dissolves more or less readily when acetic acid is added. Even in such cases there is clearly no reason to assume the presence of dibasic phosphates. Sometimes almost perfectly clear urines are obtained which give an abundant precipitate with calcium chloride. Such urines are, however, not acid to litmus, and the reason why they are clear is that they contain practically no calcium. They give no turbidity when sodium hydrate is added.

From these facts we may make the following summary: The phosphates in clear acid urine are all of the monobasic kind, and the acidity of such urines is ordinarily greater than the acidity of all the phosphates, the excess being due to free organic acids.

The current attractive, and in a measure plausible, belief that the acidity of urine is regulated by variations in the relative proportion of the two forms of "acid-phosphates" is therefore erroneous. If urine does at no time contain comparatively strong acids in the free

form, the reason is in part the variability of the ammonia formation and in part the presence of salts of organic acids. In a mixture of salts containing an excess of acids it is the weakest which will remain uncombined, and the strongest organic acids will therefore exist as salts; but if the total amount of acidity becomes abnormally great, the quality (the strength) of the free acids may change. That this may actually occur in certain pathological cases (kidney diseases) is indicated by the hydrogen ion determinations of Höber.[1]

For all ordinary studies of the acidity of urine, the direct titrations of the total acidity and of the phosphates give the necessary information. The excess of the total acidity above that calculated from the phosphates (7.1 mg. P_2O_5=1 c.c. $\frac{n}{10}$ acid) gives the total free acids present. In the tables forming a part of this paper, the data obtained by such a calculation had to be omitted for want of tabular space. The data in question can, however, easily be obtained as indicated. Here it was thought more important to show, on the one hand, the balance of the mineral acid and basic elements, and on the other, the total amount of organic acids present.

The excess of the inorganic acids expressed as "mineral acidity" decreases, it will be seen, under the influence of the starch and cream diet until it frequently becomes a minus quantity. The organic acids do not diminish to nearly so great an extent. Of special interest in connection with the organic acids are the results of July 7, in Tables IV and V when the pure starch was replaced by potatoes. The total acidity of the urine is but slightly reduced, and the total amount of organic acids eliminated is greatly increased, yet the ammonia is still considerable. This would seem to indicate that a part of the acid and ammonia formation within the organism is not affected by the alkalies of the food.

[1] Höber: Beiträge zur chemischen Physiologie, 1903, iii, p. 539.

TABLE VI.

O. F. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_g.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Feb.	Volume of urine. c.c.		Urea-N.	Ammonia N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_g.
69.6			17.3	305.0	1.58	0.49							
1	33 / 670	9.6	8.1	0.43	0.58	0.16	0.70	79.6	4.4	84.0	6.1	1.6	7.3
2	32 / 765	10.6	18.1 / 8.5	200.0 / 0.36	1.65 / 0.61	0.58 / 0.19	0.95	80.0	3.6	83.6	5.9	1.8	8.7
3	32 / 830	9.6	16.3 / 7.6	269.0 / 0.38	1.77 / 0.66	0.56 / 0.19	0.74	79.5	4.0	83.5	6.8	2.0	7.7
4	27 / 735	7.6	13.0 / 6.0	226.0 / 0.32	1.63 / 0 60	0.45 / 0.15	0.52	79.0	4.1	83.1	8.0	2.0	6.9
5	26 / 670	6.3	10.4 / 4.9	169.0 / 0.24	1.41 / 0.52	0.42 / 0.14	0.47	78.0	3.8	81.8	8.3	2.4	7.5
6	25 / 785	6.8	11.4 / 5.3	201.0 / 0.28	1.47 / 0.55	0.50 / 0.17	0.52	77.5	4.2	81.7	8.1	2.5	7.7
7	29 / 580	6.1	9.8 / 4.6	176.0 / 0.25	1.32 / 0.49	0.47 / 0.16	0.57	75.9	4.0	79.9	8.1	2.6	9.4
8	35 / 420	5.9	9.6 / 4.5	165 0 / 0.23	1.58 / 0.58	0.43 / 0.14	0.48	75.5	4.0	79.5	9.9	2.4	8.2
9	30 / 540	6.5	10.1 / 4.8	130.0 / 0.18	1.40 / 0.52	0.43 / 0.14	0.53	78.3	3.0	81.3	8.0	2.3	8.5
10	22 / 1040	6.6	10.7 / 5.0	133.0 / 0.19	1.40 / 0.52	0.48 / 0.16	0.73	76.2	2.8	79.0	7.8	2.2	11.0
11	28 / 630	4.9	7.7 / 3.6	133.0 / 0.19	1.59 / 0.59	0.45 / 0.15	0.39	73.2	3.8	77.0	12.0	3.0	8.0
12	29 / 530	3.8	5.5 / 2.6	102.0 / 0.14	1.53 / 0.57	0.40 / 0.13	0.36	68.5	3.7	72.2	14.8	3.5	9.5
13	31 / 630	4.7	7.4 / 3.4	118.0 / 0.17	1.61 / 0.61	0.41 / 0.14	0.39	71.3	3.5	75.8	13.0	2 9	8.3
14	30 / 610	5.3	7.8 / 3.7	183.0 / 0.26	1.73 / 0.63	0.44 / 0.15	0.51	70.7	4.8	75.5	12.0	2.8	9.7
15	30 / 675	5.5	9.0 / 4.2	122.0 / 0.17	1.68 / 0.62	0.42 / 0.14	0.53	76.9	3.1	80.0	11.3	2.6	6.1
67.1			9.4	139.0	1.65	0.49							
16	23 / 915	5.8	4.4	0.20	0.61	0.16	0.35	76.3	4.4	80.7	10.4	2.8	6.1

TABLE VI — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur. S_1.	S_2.	S_3.	Acidity in c.c. $\frac{N}{10}$. Total titrated.	Mineral.	Organic.	Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
1	1.80	1.46	0.12	0.22	81.1	7.8	11.1	345	−107	448	1.7	5.2	trace.	
2	1.64	1.32	0.15	0.17	80.5	9.1	10.4	278	−135	413	1.8	5.7	trace.	
3	1.63	1.30	0.16	0.17	80.0	9.6	10.4	186	−123	309	1.8	7.1	10	
4	1.01	0.83	0.08	0.10	82.2	7.9	9.9	188	−150	338	1.5	4.9	10	
5	1.14	0.91	0.10	0.13	80.0	8.7	11.3	129	−268	397	1.2	4.9	5	
6	1.22	0.91	0.13	0.18	74.6	10.6	14.8	166	−280	446	1.2	5.3	10	
7	1.09	0.82	0.11	0.16	75.2	10.1	14.7	162	304	466	1.0	4.1	trace.	
8	1.18	0.84	0.16	0.18	71.1	13.6	15.3	210	−82	292	1.1	2.9	trace.	
9	0.87	0.61	0.12	0.14	70.1	13.8	16.1	76	248	324	1.0	3.5	trace.	
10	1.01	0.76	0.12	0.13	75.2	11.8	13.0	137	−274	411	1.1	6.5	trace.	
11	0.93	0.68	0.08	0.17	73.1	8.4	18.5	101	−305	406	1.0	5.2	trace.	
12	0.97	0.70	0.10	0.17	72.2	10.3	17.5	95	−309	404	0.7	4.5	trace.	
13	1.10	0.83	0.10	0.17	75.5	9.1	15.4	76	−375	451	1.1	6.0	trace.	
14	1.21	0.90	0.12	0.19	74.4	9.9	15.3	159	334	493	1.4	5.2	trace.	
15	1.24	0.94	0.12	0.18	75.8	9.7	14.5	122	−234	356	1.3	5.8	trace.	
16	1.10	0.78	0.13	0.19	70.9	11.8	17.3	157	384	451	1.2	5.8	trace.	

TABLE VII.

DR. H. B. H. (*Normal person*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Mar.-Apr.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
90.4	*16*		*27.9*	*390.0*	*1.83*	*0.55*							
6	1740	15.2	13.0	0.55	0.68	0.18	0.62	86.1	3.6	89.7	5.1	1.2	4.0
	18		*23.6*	*337.0*	*1.90*	*0.64*							
7	1220	13.1	11.0	0.47	0.71	0.22	0.67	84.3	3.6	87.9	5.4	1.6	5.1
	21		*28.9*	*296.0*	*1.89*	*0.79*							
8	1540	15.9	13.5	0.41	0.70	0.26	1.06	84.7	2.6	87.3	4.4	1.6	6.7
	22		*28.7*	*295.0*	*1.73*	*0.70*							
9	1365	15.5	13.4	0.41	0.64	0.23	0.79	86.4	2.7	89.1	4.1	1.7	5.1
	22		*27.7*	*304.0*	*1.88*	*0.81*							
10	1460	15.0	12.9	0.43	0.69	0.27	0.68	86.2	2.9	89.1	4.6	1.8	4.5
	15		*22.8*	*247.0*	*1.66*	*0.67*							
11	1820	12.6	10.6	0.35	0.51	0.22	0.86	87.3	2.7	87.3	4.1	1.8	6.8
	17		*20.6*	*288.0*	*1.72*	*0.46*							
12	1600	11.6	9.6	0.40	0.64	0.15	0.78	83.0	3.5	86.5	5.5	1.3	6.7
	22		*19.3*	*246.0*	*1.77*	*0.63*							
13	1310	11.2	9.0	0.34	0.66	0.21	0.95	80.7	3.1	83.8	5.9	1.8	8.5
	19		*18.1*	*220.0*	*1.88*	*0.63*							
14	1220	10.5	8.5	0.31	0.70	0.21	0.84	80.5	2.9	83.4	6.6	2.0	8.0
	18		*18.1*	*279.0*	*1.81*	*0.55*							
15	1340	10.3	8.5	0.39	0.67	0.18	0.57	82.3	3.8	86.1	6.5	1.8	5.6
	22		*22.5*	*224.0*	*1.90*	*0.68*							
16	1335	12.6	10.5	0.31	0.71	0.23	0.83	83.3	2.5	85.8	5.7	1.9	6.6
	22		*20.1*	*254.0*	*1.68*	*0.54*							
17	1040	11.6	9.4	0.36	0.62	0.18	0.99	81.5	3.8	85.4	4.9	1.5	8.6
88.4	*14*		*20.1*	*356.0*	*1.86*	*0.50*							
29	2025	11.7	9.4	0.50	0.69	9.17	1.34	78.0	4.2	81.2	5.9	1.4	11.5
	22		*11.4*	*152.0*	*1.90*	*0.69*							
31	1190	7.1	5.3	0.21	0.71	0.23	0.60	75.3	3.0	78.3	10.0	3.3	8.4
	17		*10.2*	*158.0*	*1.81*	*0.53*							
1	1880	6.7	4.8	0.22	0.67	0.18	0.88	70.9	3.3	74.2	10.0	2.6	13.2
88.2	*10*		*9.7*	*145.0*	*1.78*	*0.44*							
2	2260	6.3	4.5	0.20	0.66	0.15	0.77	71.8	3.2	75.0	10.4	2.3	12.3

TABLE VII — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total titrated.	Mineral.	Organic.				
6	2.69	2.18	0.19	0.32	81.0	7.0	12.0	500	77	423	2.5	6.3	trace.	Mixed vegetarian diet. Quantity restricted.
7	2.06	1.58	0.19	0.29	76.7	9.2	14.1	445	12	457	2.1	6.1	trace.	
8	3.03	2.48	0.20	0.35	81.8	6.6	11.6	462	−49	511	2.7	6.1	trace.	
9	2.49	2.05	0.18	0.26	82.0	7.2	10.8	442	−38	480	2.4	5.5	trace.	
10	2.19	1.74	0.19	0.26	79.4	8.6	12.0	479	−134	613	2.3	6.1	trace.	
11	1.75	1.34	0.13	0.28	76.6	7.4	16.0	379	−95	474	2.3	7.4	trace.	
12	1.95	1.51	0.16	0.28	77.4	8.2	14.4	352	−122	474	2.0	7.6	trace.	
13	1.98	1.53	0.21	0.24	77.3	10.6	12.1	372	−225	597	1.8	8.1	trace.	
14	1.69	1.21	0.18	0.30	71.6	10.7	17.7	327	−156	483	1.7	8.4	trace.	
15	2.06	1.58	0.13	0.35	76.7	6.3	17.0	456	−28	484	2.1	5.4	trace.	
16	2.19	1.79	0.15	0.25	81.7	6.8	11.5	395	−69	454	2.4	7.5	trace.	
17	1.91	1.51	0.16	0.24	79.0	8.4	12.6	390	−79	469	2.0	6.2	trace.	
29	1.63	1.10	0.17	0.36	67.4	10.4	22.2	437	−89	526	1.8	8.6	trace.	All cereals and bread very much reduced.
31	1.19	0.78	0.16	0.25	65.5	13.4	21.1	224	−233	457	1.5	6.3	trace.	
1	1.27	0.79	0.13	0.35	62.2	10.2	27.6	196	−350	540	1.3	8.5	0	
2	1.30	0.71	0.14	0.45	54.6	10.7	34.7	199	−270	470	1.3	5.6	0	

TABLE VIII.

MR. N. H. G. (*Patient*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. Apr.	Volume of urine. c.c.		Urea N	Ammonia-N.	Kreatinin-N.	Uric acid N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined. N_2.
64.0	*16*	14.2	*26.1*	*308.0*	*1.20*	*0.37*	1.02	85.8	3.0	88.8	3.2	0.8	7.2
12	1880		12.2	0.43	0.45	0.12							
13	*19* 1360	13.2	*24.8* 11.6	*350.0* 0.49	*1.18* 0.44	*0.33* 0.11	0.55	88.0	3.7	91.7	3.3	0.8	4.2
14	*26* 1070	14.4	*27.7* 12.9	*347.0* 0.49	*1.10* 0.41	*0.30* 0.10	0.48	88.8	4.4	93.2	2.8	0.7	3.3
64.1	*22*	13.0	*24.7*	*302.0*	*1.06*	*0.32*	0.61	88.3	3.2	91.5	3.0	0.8	4.7
15	1020		11.5	0.42	0.39	0.11							
16	*21* 840	9.8	*18.0* 8.4	*249.0* 0.35	*1.01* 0.37	*0.23* 0.08	0.63	85.4	3.6	89.0	3.8	0.8	6.4
17	*14* 1150	7.3	*13.0* 0.61	*202.0* 0.28	*1.12* 0.43	*0.36* 0.12	0.34	83.8	3.9	87.7	5.7	1.7	4.6
19	*16* 1010	6.1	*11.1* 5.2	*125.0* 0.18	*1.08* 0.40	*0.27* 0.09	0.21	85.5	2.9	88.4	6.6	1.5	3.5
20	*16* 1130	5.8	*10.1* 4.7	*126.0* 0.18	*1.11* 0.41	*0.31* 0.10	0.39	82.0	3.0	85.3	7.1	1.8	6.8
21	*13* 1740	5.6	*9.7* 4.5	*160.0* 0.22	*1.11* 0.41	*0.36* 0.12	0.34	79.5	4.0	83.5	7.3	2.1	6.1
22	*19* 705	3.1	*4.7* 2.2	*141.0* 0.20	*1.04* 0.37	*0.27* 0.09	0.27	69.9	6.5	76.4	12.1	2.9	8.6
23	*28* 475	3.4	*4.5* 2.1	*148.0* 0.21	*1.15* 0.43	*0.25* 0.08	0.57	61.9	6.1	68.0	12.6	2.5	16.9
24	*11* 1060	4.0	*6.0* 2.8	*267.0* 0.37	*1.17* 0.44	*0.25* 0.08	0.37	68.4	9.3	77.7	10.8	2.1	9.5
25	*09* 1480	3.9	*5.7* 2.7	*243.0* 0.34	*1.18* 0.44	*0.27* 0.09	0.31	70.0	8.7	78.7	11.1	2.2	8.0
26	*13* 760	3.1	*4.3* 2.0	*167.0* 0.23	*1.14* 0.42	*0.19* 0.06	0.40	63.8	7.5	71.3	13.8	2.0	12.9
27	*12* 1270	4.0	*6.1* 2.9	*135.0* 0.20	*1.12* 0.42	*0.26* 0.09	0.42	72.0	4.9	76.9	10.4	2.2	10.5
28	*17* 610	2.6	*3.4* 1.6	*156.0* 0.22	*1.09* 0.40	*0.30* 0.10	0.30	60.3	8.4	68.7	16.0	3.8	11.5
64.0	*17*	2.9	*3.6*	*179.0*	*1.38*	*0.30*	0.38	57.5	8.4	65.9	17.6	3.4	13.1
29	580		1.7	0.25	0.51	0.10							

TABLE VIII — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur. S_1.	S_2.	S_3.	Acidity in c.c. $\frac{n}{10}$. Total, titrated.	Mineral.	Organic.	Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
12	3.19	2.60	0.29	0.30	81.5	9.1	9.4	526	210	316	3.6	7.3	10	Milk and egg diet.
13	2.88	2.18	0.32	0.38	75.7	11.1	13.2	560	277	283	3.1	5.5	60	
14	3.32	2.59	0.34	0.39	78.0	10.2	11.8	573	385	188	3.4	4.6	50	
15	2.41	1.84	0.29	0.28	76.3	12.0	11.7	481	233	248	2.8	6.5	10	
16	1.54	1.07	0.28	0.19	69.5	18.2	12.3	380	111	279	2.1	4.2	15	Starch and cream diet.
17	1.25	0.75	0.24	0.26	60.0	19.2	20.8	235	−46	281	1.5	3.1	60	
19	1.11	0.68	0.24	0.22	59.6	21.0	19.4	158	−145	303	1.5	3.1	40	
20	1.10	0.69	0.19	0.22	62.7	17.2	20.1	131	−176	307	1.6	4.8	0	
21	1.06	0.58	0.15	0.33	54.7	14.3	31.0	195	63	258	1.7	6.4	0	
22	0.69	0.37	0.09	0.23	52.2	14.5	33.3	170	−107	277	1.3	3.3	0	
23	0.58	0.37	0.09	0.12	63.8	15.5	20.7	224	−80	304	1.3	2.7	0	
24	0.68	0.40	0.07	0.21	58.8	10.4	30.8	170	−165	335	1.1	2.3	0	
25	0.75	0.42	0.09	0.24	56.0	12.0	32.0	160	−207	367	1.0	3.3	0	
26	0.66	0.37	0.09	0.20	56.1	13.7	30.3	131	−122	253	0.7	2.2	0	
27	0.73	0.36	0.13	0.24	49.3	17.9	32.8	198	−256	454	1.1	4.5	0	
28	0.61	0.26	0.19	0.16	42.6	31.2	26.2	212	−198	410	1.0	2.4	trace.	Slight fever temp. 101° F.
29	0.61	0.28	0.12	0.21	45.6	19.7	34.4	128	−125	253	0.9	2.4	trace.	

TABLE IX.

MR. N. H. F. (*Patient*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia c.c. $\frac{n}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen.	In per cent of Total Nitrogen.					
Date. June.	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined N_2.
50.1	*19*	18.3	*35.2*	*473.0*	*1.38*	*0.32*	0.57	89.9	3.6	93.5	2.8	0.6	3.1
4	1625		16.4	0.66	0.53	0.11							
5	*17*	17.0	*32.3*	*481.0*	*1.43*	*0.38*	0.54	89.0	4.0	93.0	3.1	0.7	3.2
	1840		15.1	0.67	0.51	0.13							
6	*18*	19.4	*36.6*	*481.0*	*1.51*	*0.48*	0.87	88.3	3.5	91.8	2.9	0.8	4.5
	1840		17.1	0.67	0.56	0.16							
7	*16*	18.9	*36.0*	*500.0*	*1.43*	*0.47*	0.58	89.0	3.7	92.7	2.9	0.8	3.6
	2155		16.8	0.70	0.54	0.16							
8	*19*	17.1	*32.6*	*500.0*	*1.35*	*0.45*	0.31	89.7	4.7	94.4	3.0	0.8	1.8
	1550		15.3	0.80	0.50	0.15							
58.6	*9*	11.1	*19.8*	*493.0*	*1.27*	*0.32*	0.68	83.4	5.4	88.8	4.2	0.9	6.1
9	2020		9.3	0.59	0.47	0.11							
10	*18*	7.9	*14.1*	*225.0*	*1.20*	*0.29*	0.45	83.4	4.1	87.5	5.6	1.2	5.7
	780		6.6	0.33	0.44	0.10							
11	*10*	6.4	*10.1*	*264.0*	*1.39*	*0.27*	0.63	74.7	5.8	80.5	8.1	1.6	9.8
	1245		4.7	0.37	0.52	0.09							
12	*10*	5.3	*8.9*	*258.0*	*1.31*	*0.24*	0.29	77.0	7.0	84.0	9.1	1.5	5.4
	1325		4.1	0.36	0.48	0.08							
13	*8*	5.3	*8.9*	*272.0*	*1.29*	*0.24*	0.28	77.0	7.2	84.2	9.0	1.5	5.3
	1660		4.1	0.38	0.48	0.08							
14	*8*	5.3	*8.6*	*274.0*	*1.29*	*0.21*	0.38	75.3	7.2	82.5	9.0	1.3	7.2
	1590		4.0	0.39	0.48	0.07							
15	*9*	4.7	*8.0*	*215.0*	*1.26*	*0.25*	0.20	77.6	6.4	84.0	10.0	1.8	4.2
	1170		3.7	0.30	0.47	0.08							
58.3	*11*	4.3	*6.8*	*193.0*	*1.30*	*0.18*	0.28	74.7	6.3	81.0	11.2	1.4	6.4
16	960		3.2	0.27	0.48	0.06							

TABLE IX — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur. S_1.	S_2.	S_3.	Acidity in c.c. $\frac{n}{10}$. Total, titrated.	Mineral.	Organic.	Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
4	3.62	3.14	0.23	0.25	86.7	6.3	7.0	715	305	410	4.1	5.4	40	Milk and egg diet.
5	3.85	3.31	0.24	0.30	86.0	6.2	7.8	713	258	455	4.1	5.6	20	
6	3.88	3.29	0.25	0.34	84.8	6.4	8.8	713	331	382	4.2	5.2	30	
7	3.84	3.26	0.17	0.41	84.9	4.4	10.7	638	259	379	4.2	6.6	35	
8	3.76	3.24	0.16	0.36	86.2	4.3	9.5	738	403	335	3.8	5.4	35	
9	1.53	1.06	0.16	0.31	69.3	10.5	20.2	469	250	219	3.5	3.6	trace.	Starch and cream diet.
10	1.08	0.76	0.10	0.22	70.3	9.2	20.5	368	134	234	1.8	2.6	trace.	
11	0.94	0.65	0.11	0.18	69.1	11.7	19.2	229	−20	249	1.1	2.8	trace.	
12	0.89	0.55	0.13	0.21	61.8	14.6	23.6	196	−42	238	1.1	4.0	0	
13	0.94	0.50	0.14	0.30	53.2	14.9	31.9	212	−50	262	1.0	4.3	0	
14	0.89	0.56	0.13	0.20	62.8	14.6	22.6	197	−95	292	1.0	3.4	0	
15	0.84	0.55	0.15	0.14	65.5	17.8	16.7	177	−65	242	1.0	3.0	0	
16	0.77	0.50	0.15	0.12	64.9	19.4	15.8	165	−10	175	0.9	2.7	0	

TABLE X.

MR. G. B. J. (*Patient, general paralysis*).

Weight in kilos.	Sp. gr. 1.0—.	Total nitrogen. N_2.	Urea. gm.	Ammonia. c.c. $\frac{1}{10}$ NH_3.	Kreatinin. gm.	Uric acid. gm.	Undetermined nitrogen. gm.	In per cent of Total Nitrogen.					
Date. June	Volume of urine. c.c.		Urea-N.	Ammonia-N.	Kreatinin-N.	Uric acid-N.		Urea.	Ammonia.	Urea + ammonia.	Kreatinin.	Uric acid.	Undetermined. N_2.
61.2	*18*		*25.7*	*454.0*	*1.23*	*0.26*							
8	1720	13.6	12.0	0.64	0.49	0.09	0.37	88.3	4.7	93.0	3.6	0.7	2.7
9	*19* 1780	16.5	*30.8* 14.4	*427.0* 0.60	*1.34* 0.50	*0.31* 0.10	0.94	87.1	3.6	90.7	3.0	0.6	5.7
60.8	*19*		*34.0*	*445.0*	*1.50*	*0.31*							
10	2100	17.5	15.9.	0.62	0.55	0.10	0.32	91.0	3.6	94.6	3.1	0.5	1.8
11	*16* 1195	9.8	*17.8* 8.3	*358.0* 0.50	*1.20* 0.48	*0.16* 0.05	0.50	84.3	5.1	89.4	4.9	0.6	5.1
12	*11* 1500	6.9	*11.7* 5.5	*249.0* 0.37	*1.28* 0.47	*0.18* 0.06	0.48	80.0	5.4	85.4	6.7	1.0	6.9
13	*13* 1400	6.0	*9.3* 4.3	*325.0* 0.46	*1.62* 0.60	*0.35* 0.12	0.49	72.3	7.6	79.9	10.0	1.9	8.2
14	*12* 800	4.7	*7.0* 3.3	*266.0* 0.37	*1.34* 0.49	*0.23* 0.08	0.41	70.1	7.9	78.0	10.6	1.6	8.8
15	*10* 1180	5.1	*7.7* 3.6	*392.0* 0.55	*1.48* 0.55	*0.29* 0 10	0.31	70.6	10.7	81.3	10.7	1.9	6.1
60.8	*10*		*7.0*	*233.0*	*1.38*	*0.31*							
16	1120	4.6	3.3	0.33	0.51	0.10	0.39	71.1	7.0	78.1	11.1	2.2	8.6

TABLE X — *Continued.*

Date.	Total sulphur as SO_3. "S."	Inorganic SO_3. "S_1."	Ethereal SO_3. "S_2."	"Neutral" SO_3. "S_3."	In per cent of Total Sulphur.			Acidity in c.c. $\frac{n}{10}$.			Total phosphates as P_2O_5.	Chlorine. gm.	Indican (Fehling's solution = 100).	Remarks.
					S_1.	S_2.	S_3.	Total, titrated.	Mineral.	Organic.				
8	3.28	2.88	0.22	0.18	87.8	6.7	4.5	647	229	418	3.6	5.4	40	Milk and egg diet.
9	3.17	2.71	0.22	0.24	85.5	6.6	7.9	662	370	292	3.9	5.7	40	
10	3.57	3.02	0.23	0.32	84.6	6.4	9.0	680	286	394	3.9	7.6	trace.	
11	1.32	1.00	0.16	0.16	75.8	12.1	12.1	502	229	273	2.7	2.9	trace.	Starch and cream diet.
12	1.21	0.91	0.11	0.19	75.2	9.1	15.7	270	−30	300	1.8	3.4	trace.	
13	1.38	1.01	0.13	0.24	73.2	9.4	17.4	380	56	324	1.6	3.6	0	
14	0.89	0.55	0.14	0.17	61.8	15.7	22.5	141	−32	173	0.5	2.3	0	
15	0.86	0.59	0.11	0.16	68.6	12.8	18.6	174	−231	405	0.9	2.7	0	
16	..	0.52	0.08	..	..	..	..	159	−143	302	1.1	3.2	0	

A THEORY OF PROTEIN METABOLISM.

By OTTO FOLIN.

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IN the preceding paper[1] on "Laws Governing the Composition of Urine,"[2] it was shown that urine obtained from normal persons does not necessarily exhibit any such constancy in composition as has been supposed to be the case. The analytical results recorded in that paper prove that quantitative changes in the daily protein katabolism are accompanied by pronounced changes in the distribution of the urinary nitrogen and sulphur, and that the variations occur according to laws that can be formulated with a fair degree of precision. The nature of these variations may be expressed by the following generalizations:

1. Kreatinin. The absolute quantity of kreatinin eliminated in urine on a meat-free diet is a constant quantity, different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated.

2. Uric Acid. When the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent of the uric acid nitrogen in terms of the total is therefore much increased.

3. Ammonia. With pronounced diminution in the protein metabolism (as shown by the total nitrogen in the urine) there is usually, but not always, and therefore not necessarily, a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia-nitrogen, provided that the food is not such as to yield an alkaline ash.

4. Urea. With every decided diminution in the quantity of total nitrogen eliminated, there is a pronounced reduction in the per cent of that nitrogen represented by urea. When the daily total nitrogen

[1] In vol. xiii, p. 98, line 24, read "neutral" instead of "total."

[2] FOLIN: This journal, 1905, xiii, p. 66.

elimination has been reduced to 3 gm. or 4 gm. about 60 per cent of it only is in the form of urea.

5. Inorganic Sulphates. Decided diminutions in the daily elimination of total sulphur are accompanied by reductions in the per cent of that sulphur present as inorganic sulphates. The reductions are as great as in the case of urea.

6. Neutral Sulphur. The neutral sulphur elimination is analogous to that of the kreatinin. It represents products which in the main are independent of the total amount of sulphur eliminated or of protein katabolized.

7. Ethereal Sulphates. The ethereal sulphates represent a form of sulphur metabolism which becomes more prominent when the food contains little or no protein.

In order that the reader may recall the extent of the changes in percentage composition of urine from normal persons shown by the experiments of the preceding paper, a part of Table III is here reproduced.

	July 13.	July 20.
Volume or urine	1170 c.c.	385 c.c.
Total nitrogen	16.8 gm.	3.60 gm.
Urea-nitrogen	14.70 gm. = 87.5%	2.20 gm. = 61.7%
Ammonia-nitrogen	0.49 gm. = 3.0%	0.42 gm. = 11.3%
Uric acid-nitrogen	0.18 gm. = 1.1%	0.09 gm. = 2.5%
Kreatinin-nitrogen	0.58 gm. = 3.6%	0.60 gm. = 17.2%
Undetermined nitrogen	0.85 gm. = 4.9%	0.27 gm. = 7.3%
Total SO_3	3.64 gm.	0.76 gm.
Inorganic SO_3	3.27 gm. = 90.0%	0.46 gm. = 60.5%
Ethereal SO_3	0.19 gm. = 5.2%	0.10 gm. = 13.2%
Neutral SO_3	0.18 gm. = 4.8%	0.20 gm. = 26.3%

It is clear that the laws governing the composition of urine represent only the effects of other more fundamental laws governing the katabolism of protein in the animal organism. From the variations in the percentage composition of urine described by the above generalizations, it would seem therefore that some conclusions may be drawn in regard to the nature of protein metabolism. It is my purpose in the present paper to attempt an interpretation of protein metabolism on the basis of observed variations in the percentage composition of urine.

We have at present two fundamentally different theories concerning the nature of protein metabolism, namely, that of Pflüger and that of Voit. The theory of Pflüger is essentially a modification of an

earlier one advanced by Liebig, and is very old. The theory of Voit was first formulated in 1867, after the original theory of Liebig had become untenable, and for a long time Voit's theory enjoyed almost universal acceptance, although it, too, had to be modified in order to be consistent with the facts brought out by Pflüger. Since 1893 Voit's theory may be said to have lost ground. In that year Pflüger[1] published an exceedingly searching criticism of nearly all the facts which Voit had advanced in favor of his theory, and showed that they were either erroneous or capable of a different interpretation. This criticism, accompanied as it was by the experiments of Schöndorff, has never been refuted.

The two theories are briefly as follows: According to Voit, the protein of the absorbed food passes through the blood to the different tissues and cells, and is there katabolized under the influence of the living protoplasm, but without first becoming an integral part of the latter. Voit's fundamental conception seems to me to be that the living protoplasm is in a state of suspension, the "circulating protein" is in solution, and the chemical decompositions that constitute protein katabolism take place only in solution. The small amount of living protoplasm which dies in the course of twenty-four hours is at first only dissolved, thereby becoming a part of the circulating protein derived directly from the food.

Pflüger, on the other hand, believes that there is a very decided chemical difference between circulating protein and living protoplasm. The former is comparatively stable toward oxidizing reagents, while the latter is in a very unstable equilibrium and is particularly susceptible to oxidation. All the protein katabolized is first transformed into bioplasm, becomes an integral part of the living tissue, and only as such undergoes the oxidation that is supposed to constitute the most fundamental chemical decomposition of protein katabolism. It must be stated, however, that Pflüger can scarcely be represented as unqualifiedly committed to this theory. He is generally so represented, but chiefly on account of his extremely hostile and uncompromising attitude toward the theory of Voit. In the latter part of the paper published in 1893 (pp. 414–419) he practically admits the possibility that a certain amount of protein may be katabolized in solution, *i. e.*, without having acquired the structure of living protoplasm.

The chief, if not the only positive evidence against Voit's theory.

[1] PFLÜGER: Archiv für die gesammte Physiologie, 1893, liv, p. 334.

and in favor of Pflüger's, rests upon the experiments of Schöndorff.[1] These showed that when the blood from a starving dog was passed through the hind legs and liver of a well fed dog, the urea contents of the blood were increased, while no such increase was found when blood, whether of starved or well fed dogs, was passed through the hind legs and liver of a dog that had previously starved for several days. This result obtained from sixteen experiments has been generally accepted as proving the point which Pflüger and Schörndorff intended it to prove, namely, that it is the state of nutrition in muscle cells and not the circulating protein which is the determining factor in the protein katabolism.

It seems to me, however, that the evidence furnished by these experiments is by no means unassailable. After a dog has starved several days his protein katabolism has of course been reduced to an extremely low level. When such a dog has been bled to death, and his legs are washed out with normal salt solution, it does not seem very peculiar that the passing through of defibrinated blood should not revive the protein katabolism in the legs to a demonstrable extent. Schöndorff's well fed dogs, on the other hand, had been taking enormous quantities of meat, 1000 gm. or 1200 gm. a day, and special efforts were made to have them at the height of digestion when killed. In these dogs, therefore, we must assume, according to both Voit and Pflüger, that the protein katabolism taking place in the muscle tissues of the legs at the time of the killing was enormously increased above that in the tissues of the starved animals. That a certain amount of nitrogenous katabolism products should be obtained on passing blood through the legs of such animals seems to me by no means to prove Pflüger's point.

The evidence obtainable from these experiments depends not on whether there was an increase in the urea contents of the blood passed through the muscles of the well fed animals, but on the quantity of that increase. Schöndorff does give the increase in terms of the urea present in the blood at the beginning of each experiment and thereby gets figures which seem very striking indeed (100 per cent or more), but that is only because the original urea content of the blood was insignificant. In his last experiment (No. 16, p. 481), however, Schöndorff gives in addition the absolute quantity of urea which he believes must certainly be held to represent the protein katabolism occurring in the muscles of the dog while the blood was

[1] SCHÖNDORFF: Archiv für die gesammte Physiologie, 1893, liv, p. 420.

passed through. This dog weighed 18.5 kgm. During the last four days preceding the experiment he had taken 1200 gm. meat per day, and during the last nine hours immediately preceding the experiment he had taken 700 gm. of meat. In the course of four and a half hours the blood was passed through the legs sixteen times. The urea obtained amounted to 53 mg., the urea nitrogen therefore to 25 mg. This dog had during the preceding four days katabolized in the neighborhood of 35 gm. of nitrogen per day. Expressed in terms of this nitrogen, the two hind legs had during four and a half hours katabolized less than one-tenth of 1 per cent! Considering the numerous sources of error and uncertainty necessarily attached to an experiment of this kind, it seems very strange that the extraction of 25 mg. of urea-nitrogen from the hind legs of a dog killed while engaged in digesting 700 gm. of meat should be accepted as proving not only that protein katabolism did occur during the experiment, but also that it occurred in the bioplasm and not in the circulating protein.

The chief positive evidence in favor of Voit's theory not disproved by Pflüger is the well known fact that extremely large quantities of food protein are more or less completely katabolized in the course of a few hours. It is considered incredible that such enormous building up of bioplasm, accompanied by immediate destruction of it, can take place in so short a time, especially since the previous state of nutrition of the organism has practically nothing to do with it. While the theory of Voit is no longer so generally accepted as was once the case, it undoubtedly still has a greater number of adherents than has the theory of Pflüger.

The analytical data obtained in connection with the metabolism experiments described in the preceding paper seem to me to have a direct bearing on this general fundamental question concerning protein metabolism. No fundamental theory concerning that metabolism can be accepted unless it can be made to harmonize with the laws governing the composition of urine. If heretofore no objection has been raised against either Voit's or Pflüger's theory on such grounds, it is only because the accepted views concerning the composition of urine agree sufficiently well with either. At the time when these theories were advanced, nothing inconsistent with their correctness was known in connection with the composition of urine, nor are the views concerning that composition to-day essentially different from what they were then, *i. e.*, quantitative changes in the

total protein metabolism have been supposed to have no appreciable effect on the percentage composition of the urines representing such metabolism. But this is clearly not correct. Quantitative changes in the protein metabolism, or in other words, changes in the total amount of nitrogen and of sulphur eliminated in the urine, are accompanied by pronounced and unmistakable changes in the percentage composition of the latter.

But this fact constitutes, it seems to me, definite proof that neither the theory of Voit nor that of Pflüger can be correct.

We have seen from the tables that the composition of urine, representing 15 gm. of nitrogen, or about 95 gm. of protein, differs very widely from the composition of urine representing only 3 gm. or 4 gm. of nitrogen, and that there is a gradual and regular transition from the one to the other. To explain such changes in the composition of the urine on the basis of protein katabolism, we are forced, it seems to me, to assume that that katabolism is not all of one kind. There must be at least two kinds. Moreover, from the nature of the changes in the distribution of the urinary constituents, it can be affirmed, I think, that the two forms of protein katabolism are essentially independent and quite different. One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no kreatinin, and probably no neutral sulphur. The other, the constant katabolism, is largely represented by kreatinin and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total katabolism is reduced, the more prominent become these representatives of the constant katabolism, the less prominent become the two chief representatives of the variable katabolism.

The fact that the urea and inorganic sulphates represent chiefly the variable katabolism does of course not preclude the possibility that they also represent to some extent the constant katabolism; but I have reason to believe that it is possible to plan feeding experiments which will yield urines containing very much smaller per cents of these two constituents than I have yet obtained. We know from the experiments of Sivén that it is possible to reduce the total protein katabolism still more, and I am confident that in such cases the per cent of urea-nitrogen will sink still lower, and that the nitrogen of the other constituents, particularly of the kreatinin, will again show a corresponding increase.

If there are two distinct forms of protein metabolism represented

by two different sets of waste products, it becomes an exceedingly interesting and important problem to determine, if possible, the nature and significance of each. The fact that the kreatinin elimination is not diminished when practically no protein is furnished with the food, and that the elimination of some of the other constituents is only a little reduced under such conditions, shows why a certain amount of protein must be furnished with the food if nitrogen equilibrium is to be maintained. It is clear that the metabolic processes resulting in the end products which tend to be constant in quantity appear to be indispensable for the continuation of life; or, to be more definite, those metabolic processes probably constitute an essential part of the activity which distinguishes living cells from dead ones. I would therefore call the protein metabolism which tends to be constant, *tissue* metabolism or *endogenous* metabolism, and the other, the variable protein metabolism, I would call the *exogenous* or intermediate metabolism.[1]

The endogenous metabolism sets a limit to the lowest level of nitrogen equilibrium attainable. Just where that level is fixed will depend on how much, if any, urea is derived from the same katabolic processes that produce the kreatinin. If this can be determined, we shall have a formula expressing more or less definitely the point of lowest attainable protein katabolism, because at such a point the percentage composition of the urine should be practically constant.

The total nitrogen eliminated when this constant composition of the urine has been reached will indicate the lowest attainable level of nitrogen equilibrium. Whether or no such a level can actually be attained, or whether a certain amount of protein must not always fall prey to the exogenous metabolism, can only be settled by a great deal of experimental work.

In connection with such work the significance and the importance of the exogenous protein metabolism might also be settled. Is any of it indispensable, and if so, how much? The analytical data recorded in the preceding paper make it clear that protein sufficient to maintain the endogenous protein metabolism is indispensable. And the chemistry of the exogenous protein metabolism ought also to furnish evidence as to its importance to the organism. A fair amount of such chemical evidence is, I believe, already available, but before con-

[1] These names are chosen because they have already been frequently used as indicating the origin of special metabolism products.

sidering it, or rather in connection with it, I desire once more to refer briefly to the above mentioned theories of Pflüger and Voit.

The question that naturally arises is, do these two forms of protein-metabolism, *i. e.*, exogenous and endogenous metabolism, represent the views held by Voit and Pflüger? Does Pflüger's view cover the tissue, the endogenous metabolism, and does Voit's view correctly represent the variable, the exogenous metabolism?

The endogenous metabolism is of course more or less identical with the protein metabolism of Pflüger, since it represents the decomposition of living protoplasm. Viewed more in detail it is, however, different, because it yields end products in proportions entirely different from those which Pflüger knew.

The exogenous protein metabolism has, I believe, nothing in common with Voit's metabolism of circulating protein.

The difference between the theory of Voit and that of Pflüger is not unimportant; but on a matter of far greater importance they agree, namely, on the universally accepted assumption that the entire katabolism of protein takes place in the same tissues and by means of katabolic processes similar to those which bring about the decomposition of the non-nitrogenous food, *i. e.*, the fats and carbohydrates. In other words the protein katabolism is supposed to be essentially an oxidation, and the greatest amount of protein katabolism is supposed to take place where the greatest amount of oxidation occurs, *i. e.*, in the muscles.

But the nitrogen of protein is invariably linked to carbon on the one hand and to hydrogen on the other, *i. e.*, it exists as amido or imido groups, and from a chemical point of view no oxidation is necessary for the splitting off of the nitrogen of such groups.

Such splittings are more easily accomplished by hydrolytic reactions than by oxidations. For example, in a $\equiv CNH_2$ or $= C = NH$ group, the nitrogen can be split off as ammonia by the simple addition of a molecule of water. There is, therefore, *a priori* no reason for assuming that the katabolism of the protein-nitrogen is brought about by the same chemical processes as those which decompose the fats and the carbohydrates.

Have we any valid reason for believing that these two sets of chemical decompositions occur simultaneously or even in the same tissues? I do not think that we have. On the other hand, when this question is once fairly raised there is, it seems to me, considerable evidence against it.

The fact that urea, the chief representative of the exogenous protein metabolism, is not to be found in the muscles (except in infinitesimal traces) acquires a new significance from this point of view. The absence of urea in muscles where the greatest amount of general katabolism takes place is to this day an unexplained fact. Many attempts at explanation have been made. It has been thought that the protein decomposition in the muscles does not proceed as far as the urea, but only produces certain precursors of it which are afterwards elaborated in the liver into urea. But the only precursor which is found in quantities that could be considered at all adequate is kreatin, and it has been shown that the liver is not capable of converting kreatin into urea. Moreover the perfect constancy in the kreatinin elimination shown in these experiments under such different conditions excludes, it seems to me, positively the possibility that the kreatin produced in the protein katabolism of the muscles is afterwards converted into urea. The muscle kreatin is eliminated as kreatinin, and the presence of such considerable quantities of kreatin in the muscles, coupled with the absence of urea, as well as of any known precursor of urea, constitutes (in the light of the manifestly different laws governing their elimination) exceedingly strong evidence against the view that the nitrogen katabolism represented by urea takes place to any considerable extent in the muscles. If this is so, it explains why the experiments of Schöndorff referred to above gave practically negative results.

But if the nitrogen katabolism represented chiefly by urea does not occur in such important tissues as the muscles, then we are practically forced to the conclusion that this nitrogen katabolism is brought about by special chemical processes in special places, and that this katabolism is not of any such general fundamental importance as is the katabolism that yields kreatinin. Several other facts point in this direction, and I know of no facts contradicting it. We know that in the protein digestion ammonia is formed directly by the action of the proteolytic ferments, and we know that the liver is capable of converting that ammonia into urea. That the ammonia formed during digestion is not recombined into protein compounds we know from the investigations of Nencki,[1] which showed that the blood coming from the intestines at the height of digestion contains two or three times as much ammonia as the blood on the other side of the liver. My

[1] Nencki and Zaleski: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 206.

own ammonia determinations published two years ago have confirmed this result.[1] Here we have therefore positive evidence that a part of the food nitrogen is eliminated as urea without having ever passed through the muscles. The recent discovery by Cohnheim[2] of erepsin in the mucous membrane of the intestines, points in the same direction. Cohnheim was looking for an enzyme which should be able to recombine peptones or albumoses into albumin, but found instead an enzyme which splits them still further, *i. e.* into amido acids. The work of Kutscher had previously showed that trypsin is capable of completely splitting protein products into relatively small amido acid molecules.

The prevailing idea that these splitting products before entering the blood are recombined into albuminous bodies has never been proved. The chief attempts made in this direction have not taken the deep seated decompositions of trypsin and erepsin into account; and the failure to find peptones or proteoses has been accepted as showing that these products are at once in the mucous membrane converted into albumin and only as such enter into circulation. To be sure, Kutscher also failed to find the amido acids in the blood, and was himself therefore forced to assume, in accordance with earlier investigators, that they must be recombined into albuminous products before they get through the mucous membrane of the intestines.[3] Bergmann and Langstein[4] were also unable to show that protein digestion in dogs is always accompanied by an increase of non-albuminous nitrogen in the blood, although their figures indicate that this is usually the case.

It seems to me that the experimental conditions chosen by Bergmann and Langstein are in a measure inconsistent with the theoretical part of their paper. In their experiments they determine the non-coagulable nitrogen in the blood of fasting dogs and of dogs at the height of digestion in the hope of finding a demonstrable difference due to digested food protein But they use only 10 c.c. or 15 c.c. of blood for the determination of this nitrogen, yet they show by calculation that 30 gm. of nitrogen could be absorbed from the intestines of a man in the course of four hours with-

[1] FOLIN: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 174.

[2] COHNHEIM: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 451.

[3] KUTSCHER und SEEMANN: Zeitschrift für physiologische Chemie, 1902, xxxiv, p. 529.

[4] BERGMANN and LANGSTEIN: Beiträge zur chemischen Physiologie und Pathologie, 1904, vi, p. 27.

out the total increase of nitrogen in the blood amounting to more than 5 mgm. per 100 c.c. Their calculations are, however, equally applicable to dogs, and it is therefore not in any sense remarkable that they could not always demonstrate such an increase in 10 c.c. or 15 c.c. of blood. That they actually did find such an increase in some cases is, however, decidedly interesting.

The failures that have attended the attempts to show that protein digestion is accompanied by an increase of the non-proteid nitrogen of the blood must as yet be ascribed to unfavorable conditions or to lack of sufficient accuracy in the technique. The determinations of the ammonia show that there must be such an increase.

The chief reason why the nitrogenous splitting products produced by the digestive enzymes are universally assumed to be reconverted into albumin is the teleological one. The food proteins are tissue builders and the organism must therefore not waste them. The fact that the muscle tissues of normal men do not increase when the protein of the food is increased, but that all of the nitrogen of such protein is at once eliminated, has not been sufficiently considered in this connection. The only adequate teleological explanation of this fact is that this nitrogen is not needed for the building of new tissues. It is not needed because the organism cannot enlarge indefinitely, and because after it has attained its full growth the daily waste of tissue is small. Yet when more nitrogen than the organism needs is furnished with the food, we find that the protein containing it is still absorbed up to the limit of the digestive capacity. What is the teleological explanation of this fact?

In order to explain this phenomenon Pflüger and Voit have advanced the now generally accepted hypothesis that the organism uses protein by preference, even when an abundance of fats and carbohydrates is available. But this hypothesis again is almost indissolubly connected with the assumption that it is because of its nitrogen that protein is preferred. Yet nobody, not even Voit, believes that a diet giving 25 gm. of urinary nitrogen is to be preferred for the average man to a diet giving 15 gm. or 16 gm. of such nitrogen. Why this arbitrary limitation? It is not indicated by the chemistry of protein metabolism. When a man has established a nitrogen equilibrium, whether on 10 gm. or on 20 gm. of nitrogen, an increase of nitrogen in the food is immediately followed by an increase in the elimination. At 20 gm. as at 10 gm. the organism seems therefore by preference to use any additional protein that is furnished with the food.

There is abundant evidence that extensive hydrolytic decomposition of protein occurs in the digestive tract, and from the presence of the enzymes, pepsin, trypsin, and erepsin, we must assume that the decompositions which they can accomplish are for the advantage of the organism. The usefulness of the proteolytic enzymes has always been admitted, but their usefulness, it has been thought, must be restricted to the mere solution of coagulated proteins. This restriction is probably in a large measure due to the influence of Liebig, who held first that protein is the sole source of muscular work and, secondly, that the animal organism must obtain all its protein in preformed condition because it is unable to synthesize it out of simple crystallizable products. The first idea is now no longer believed to be correct, and the second is seriously questioned; but the formation of amido acids in protein digestion is still considered as so much waste of valuable material, because the organism is still supposed to need those products in the form of protein.

It is clear that the teleological argument in favor of the immediate recombination of protein digestion molecules into albumin has no more value than the same argument earlier advanced against the view that the protein digestion could give rise to any considerable quantities of crystallizable amido acid products. Such an argument may be applicable to a part of the protein metabolism, but clearly cannot be true of the whole. The well established fact that the animal organism tends to maintain nitrogen equilibrium within such tremendously wide limits as those indicated on the one hand by "forced" feeding, and on the other by the low nitrogen experiments, is utterly inconsistent with any such teleological argument in favor of albumin formation. Such an extensive formation of Voit's "circulating protein," followed by a second immediate decomposition and elimination as urea, seems to me almost if not quite as improbable as the corresponding formation and decomposition of Pflüger's organized protoplasm.

According to the views here presented on the other hand, only a small amount of protein, namely, that necessary for the endogenous metabolism, is needed. The greater part of the protein furnished with standard diets like Voit's, *i. e.* that part representing the exogenous metabolism, is not needed, or, to be more specific, its nitrogen is not needed. The organism has developed special facilities for getting rid of such excess of nitrogen so as to get the use of the carbonaceous part of the protein containing it. The first step in this

process is the decomposition of protein in the digestive tract into proteoses, amido acids, ammonia, and possibly urea.[1] The hydrolytic decompositions are carried further in the mucous membrane of the intestines, and are completed in the liver, each splitting being such as to further the formation of urea.

In these special hydrolytic decompositions, the result of which is to remove the unnecessary nitrogen, we have an explanation of why and how the animal organism tends to maintain nitrogen equilibrium even when excessive amounts of protein are furnished with the food. This excess of protein is not stored up in the organism, as such, because the actual need of nitrogen is so small that an excess is always furnished with the food, except, of course, in carefully planned experiments. The ordinary food of the average man contains more nitrogen than the organism can use, and increasing the nitrogen still further will therefore necessarily only lead to an immediate increase in the elimination of urea, and does not increase the protein katabolism involved in the kreatinin formation any more than does an increased supply of fats and carbohydrates.

The normal human organism can be made at almost any time to store up fats and carbohydrates. The katabolism of these products consists chiefly of oxidation, a decomposition which sets free large quantities of heat which can be converted into mechanical energy useful to the organism. The hydrolytic removal of nitrogen from the protein involves by comparison a very small transformation of energy and yields a non-nitrogenous rest of great fuel value. This non-nitrogenous rest derived from protein may partly be directly transported to the different tissues, and thus at once supply oxidative material where needed, but in all probability is partly converted into fats, or at least into carbohydrates, and then becomes subject to the laws governing the katabolism of these two groups of food products.

I fully believe that the view here developed concerning protein metabolism is substantially correct. More detailed studies may necessitate minor modifications and additions. For example, it may well be that the digestive tract and the liver are not alone sufficient to split off all the unnecessary nitrogen. Other glands may play a part, for we know that proteolytic enzymes resembling trypsin are present in such glands. And it may be that a detailed study of the urines of carnivorous animals, as the cat and the dog, may indicate a somewhat

[1] KOSSEL and DAKIN: Zeitschrift für physiologische Chemie, 1904, xlii, p. 181.

different condition. We know in fact that these animals can store up great quantities of protein (in the form of increased muscle substance?). Their endogenous metabolism may therefore be much less constant than that of man. The urines of such animals contain nitrogenous products not found in human urine. Their protein katabolism is therefore qualitatively as well as quantitatively more or less different. Similarly with reference to herbivorous animals. They also yield nitrogenous waste products quantitatively quite differently distributed than are those found in man, and detailed studies of those products under different conditions will undoubtedly throw additional light on the whole subject here under discussion. But I do not think that such studies will materially affect my main contention that protein katabolism in the animal body is of two kinds, that one of these only is true tissue katabolism, and that the other consists of a preliminary removal of unnecessary nitrogen by means of hydrolytic splitting processes which are distinct and separate from the decompositions associated with subsequent oxidations.

The exogenous or intermediate protein katabolism is here conceived as consisting of a series of hydrolytic splittings resulting in a rapid elimination of the protein-nitrogen as urea. This view does not, however, preclude the possibility that a certain amount of oxidation is associated with this form of katabolism. In fact, we must assume that this is to some extent the case. The hydrolytic removal of the nitrogen would leave hydroxyl groups in the positions previously occupied by the amido groups. But since it is almost absolutely certain that the non-nitrogenous rest is in part converted into carbohydrates, a certain amount of oxidation and of so-called aldol condensation must constitute an important part of the chemical transformations necessary to the formation of glucose.[1] The splitting off of the protein-nitrogen as ammonia, and its combination with carbon dioxide, and subsequent conversion into urea, demands no oxidation. But just as oxidation is necessary for the formation of carbohydrates out of the non-nitrogenous rest, so a certain amount of oxidation is necessary to bring about the conversion of the protein-sulphur into sulphuric acid.

[1] A full discussion of this question cannot be attempted here. That the animal organism can form glucose out of protein derivatives containing no preformed carbohydrate groups is wellnigh positively demonstrated. See LEO LANGSTEIN'S interesting article on this subject in Ergebnisse der Physiologie, iii, part 1, p. 453 (1904). See also LÜTHJE: Archiv für die gesammte Physiologie, 1904, cvi, p. 160.

Deductions Concerning Some More Special Problems in Metabolism.

The theory developed on the preceding pages concerning protein metabolism would cause a number of important problems to appear in a new light.

Nitrogen equilibrium. — We have already seen how the theory explains the persistent tendency on the part of the organism to maintain nitrogen equilibrium, even when this involves the formation of excessive quantities of urea. A rather interesting and instructive illustration of this phenomenon is shown in the third periods of the feeding experiments represented by Tables II–V of the preceding paper. The period represents a return to a nitrogen-rich diet after the subjects had been losing considerable nitrogen during a week or more when on a diet containing less than 1 gm. of nitrogen.

On the very first day that nitrogen was obtained with the food, the elimination of this element was more than doubled, and on the second day more than three times as much nitrogen was eliminated as on the last day of nitrogen starvation. Sivén observed the same phenomenon, and found that after having lost 32.4 gm. of nitrogen on a protein-poor diet, he regained only 20.6 gm. in the course of thirteen days on a diet very rich in nitrogen. Sivén interprets this as speaking against Pflüger's and in favor of Voit's theory of protein katabolism.[1] He explains the phenomenon by assuming that the loss of nitrogen represents loss of muscle substance, and that the subsequent rebuilding of the lost muscle tissue is a slow process. But the detailed analytical work carried out in connection with my feeding experiments clearly indicates that the loss of nitrogen does not represent loss of muscle tissue, and Sivén's uric acid determinations certainly point in the same direction. I would offer the following explanation: All the living protoplasm in the animal organism is suspended in a fluid very rich in protein, and on account of the habitual use of more nitrogenous food than the tissues can use as protein the organism is ordinarily in possession of approximately the maximum amount of reserved protein in solution that it can advantageously retain. When the supply of food protein is stopped, the excess of reserve protein inside the organism is still sufficient to

[1] Sivén: Skandinavisches Archiv für Physiologie, 1901, xi, p. 320.

cause a rather large destruction of protein during the first day or two of protein starvation, and after that the protein katabolism is very small, provided sufficient non-nitrogenous food is available. But even then, and for many days thereafter, the protoplasm of the tissues has still an abundant supply of dissolved[1] protein, and the normal activity of such tissues as the muscles is not at all impaired or diminished. When 30 gm. or 40 gm. of nitrogen have been lost by an average-sized man during a week or more of abstinence from nitrogenous food the living muscle tissues are still well supplied with all the protein that they can use. That this is so, is indicated on the one hand by the unchanged kreatinin elimination, and on the other by the fact that one experiences no feeling of unusual fatigue or of inability to do one's customary work. Because the organism at the end of such an experiment still has an abundance of available protein in the nutritive fluids, it is at once seemingly wasteful with nitrogen when a return is made to nitrogenous food. This is why it only gradually, and only under the prolonged pressure of an excessive supply of food-protein again acquires its original maximum store of this reserve material.

Standard diets. — If the interpretation just given for the phenomenon of nitrogen equilibrium is correct, it constitutes at the same time a definite reason why the so-called standard diets are unnecessarily rich in protein. Nitrogen enough to provide liberally for the endogenous metabolism and for the maintenance of a sufficient supply of reserve protein is shown to be necessary. But it ought neither to be necessary nor advantageous for the organism to split off and remove large quantities of nitrogen which it can neither use nor store up as reserve material. In the case of carnivorous animals, the uncertainty of the food supply has evidently led to the development of the capacity to store a certain amount of protein in the form of increased muscle substance, but in man this capacity seems not to exist. The slowness with which the normal human organism stores nitrogen after having lost only very moderate amounts, does not mean that the human organism can replace lost muscle tissue only slowly and with difficulty. When the organism really has suffered a loss of such tissue, as, for example, during typhoid fever, we know that during convalescence there is an astonishingly rapid recovery of weight

[1] Whether such solution is colloidal or otherwise, and whether such dissolved reserve protein is stored in the blood and lymph, or also, as seems to me highly probable, in the cellular fluids, is immaterial in this connection.

and a correspondingly extensive retention of nitrogen.[1] The loss of 25 gm. or 30 gm. of nitrogen, due to the withdrawal of nitrogenous food, does not involve loss of muscle substance, 1st, because the endogenous metabolism is not affected; 2d, because it is not accompanied by loss of strength and ability to do work; 3d, because from a teleological standpoint it seems highly improbable that the consumption of nitrogenous material in a system consisting of living protoplasm immersed in a highly nitrogenous solution should be at the expense of the protoplasm rather than at the expense of the solution. The chief reservoir for nitrogenous reserve material in the human organism seems therefore to be the unorganized protein in the fluid media rather than the protoplasm of the muscles. This is why there is normally in man a comparatively sharp limit to his capacity for storing protein, and why all excess of nitrogen given with the food is promptly eliminated.

The immediate elimination of the greater part of the nitrogen contained in 118 gm. of protein by means of the exogenous katabolism would seem to constitute very strong evidence in favor of the view that the protein so katabolized can without harm, if not with advantage, be replaced by an equivalent quantity of carbohydrates.

The above interpretation of the phenomenon of nitrogen equilibrium is based on the generally accepted view that the human organism ordinarily cannot be made to increase its store of nitrogen. Recent experiments by Lüthje[2] and others[3] have, however, shown that this view is not strictly correct. By means of short feeding experiments with extraordinarily large quantities of protein (40 gm. to 60 gm. of nitrogen or more per day) or with diets containing enormous fuel value (70 calories per kilo of body weight) the human organism can evidently be made to retain for a time considerable extra quantities of protein. These experiments are highly interesting, but they seem to me to have less bearing on the question of nitrogen equilibrium than may at first sight appear to be the case. There must be a limit to the amount of food, particularly of protein, which the organism can dispose of by means of normal physiological processes. The peculiar feature of such forced feeding experiments is the introduction of *enormous* quantities of protein for a *short* time (40–60

[1] Lüthje and Berger: Deutsches Archiv für klinische Medizin, 1904, lxxi, p. 278.

[2] Lüthje: *Loc. cit.*

[3] See Kaufmann: Zeitschrift für diatetische und physikalische Therapie, 1903–4, vii, p. 355. See also Albu: Berliner klinische Wochenschrift, 1904, xlvii, p. 1228.

gm. of nitrogen means from eight to fifteen times as much as is actually needed for the maintenance of nitrogen equilibrium). We have but to assume that the digestive tract is able to handle such quantities for a few days, but that the subsequent physiological processes involved in the katabolism are more limited, and we are forced to conclude that the organism is then compelled to dispose of a part of such nitrogen in a manner more or less abnormal. The fact that a part of such excess of nitrogen is retained for a time at least, is interesting, but this may indicate only that the organism is less damaged by retaining it in one form or another than by eliminating it in pathological forms through the kidneys, as would be the case if it is flooded with protein derivatives which it cannot use.

Detailed study of the nitrogen that is eliminated under such extraordinary conditions might throw some light on the processes involved. If 50 gm. of urinary nitrogen is eliminated in twenty-four hours from a diet containing no meat, this nitrogen should contain not less than 96 per cent of the total as urea and ammonia in case the physiological katabolic processes involved are adequate. If they are not adequate, if the excess nitrogen is stored because it cannot be normally eliminated, then we should probably (though not necessarily) find that less than 96 per cent is present as urea and ammonia, and that the deficit is accounted for, not by an increase of kreatinin and uric acid, but by the presence of other nitrogenous constituents not ordinarily present in human urine. In other words the inadequacy of the physiological katabolic processes may result in the escape of some nitrogen without the preliminary conversion into urea.

The two chief arguments which have been advanced in justification of large amounts of protein (118–130 gm.) are first that people who can afford it actually do frequently consume such large quantities of protein, and secondly that Voit and his co-workers thought they had shown that nitrogen equilibrium cannot be permanently maintained on smaller amounts.

The first argument has, however, no more value than the same argument would have if applied to the question of the daily use of wine at the table. Like most statistical arguments this one is capable of quite different interpretations. The second argument has carried incomparably greater weight. It has repeatedly been shown that nitrogen equilibrium can be maintained on less than one-third the nitrogen demanded by the standard diets; but Voit came long ago to a different conclusion, and the experiments of the more recent investigators have not been able to awaken any general doubt as to the

substantial correctness of the conclusions of Voit. Even the men who have successfully made low nitrogen equilibrium experiments have hesitated to draw conclusions essentially different from those of the older master. "Doch wäre es sicherlich übereilt," says Sivén, "von einem Ueberschusse zu sprechen bevor man den Zweck kennt, wozu der Organismus das Eiweiss verwendet, welches es täglich verzehrt. Was wissen wir darüber"?

"Wir wissen, dass das Eiweiss in der Nahrung für die Muskelarbeit nicht notwendig ist; wir wissen dass ein gewisses Minimum nicht unterschritten werden darf, ohne dass der Organismus darunter leidet, aber wir wissen nicht weshalb dem so ist; wir wissen nicht, welchen vitalen Processen das Eiweiss im Allgemeinen, und speciell diese verhältnissmässig geringe Eiweissmenge dient."[1]

The appearance of Chittenden's book a few weeks ago, on Physiological Economy in Nutrition, seems, however, to me to indicate the beginning of the end of Voit's nitrogen-rich standards of diet. Here we find feeding experiments numerous enough and prolonged enough to constitute incontrovertible evidence against the necessity of conforming to the standards of Voit. And Chittenden moderately but firmly takes the position indicated by the experiments that the current dietary standards can safely be divided at least by two as far as their protein contents are concerned.

The conclusions here derived from detailed studies of the composition of urine corresponding to different amounts of protein katabolism are therefore in harmony with recent low nitrogen experiments made purely from the nitrogen equilibrium standpoint. Theoretically, as well as practically, 118 gm. of protein have therefore been shown to be far in excess of the needs of normal man.

Can anything more be said? Can it be shown that such large quantities of protein are not only unnecessary but in addition detrimental? This is undoubtedly a much more difficult question to answer. It has frequently been argued that the excessive production of uric acid, xanthin bases, kreatin, etc., in short, of the so-called extractives, is responsible for the gouty tendencies so common among people known to consume large quantities of protein. This may be true. There may be, probably is, some genetic relation between the production of uric acid and of gout, but this cannot be admitted as showing or indicating that the use of excessive quantities of protein is responsible for it. Such arguments can evidently be applied only

[1] Sivén: *Loc. cit.*, p. 332.

to meat eating, because the analytical data recorded in the preceding paper show that the excess of protein consumed is converted into harmless urea. The protein does not lead to an excessive production of uric acid, and does not at all increase the kreatinin. Crude meat, on the other hand, contains considerable amounts of such extractives or substances out of which they can be formed, and since meat constitutes the chief source of concentrated protein food, the argument may have a certain practical value. But from a purely scientific point of view it has no bearing on the question raised at the beginning of this paragraph. Yet it would seem that since 118 gm. or 130 gm. of protein are not necessary, the daily use of such large amounts may sooner or later lead to metabolism disorders, and thus be detrimental.

The prevailing views of the phenomenon of nitrogen equilibrium are based on the supposition that the organism uses protein by preference, instead of fats and carbohydrates, and that protein is therefore the kind of food most suitable to the needs of the organism. This view, if pushed to its logical conclusion, is equivalent to saying that the more protein the food contains, the better it is; in other words, there can be no such thing as " luxus-consumption " of protein. But Pflüger is, as far as I know, the only one who in recent times has held this extreme position. To my mind this interpretation of the protein consumption is simply a more exact statement of the popular idea that large quantities of meat increase the strength and endurance of healthy men.

In the light of the theory developed in this paper concerning the double nature of protein metabolism and the explanation of the phenomenon of nitrogen equilibrium, the following objection can perhaps be made to the use of large quantities of protein.

The excess of nitrogen furnished with the food is normally quickly converted into urea and eliminated, and is, therefore, normally harmless. The continuous excessive use of protein may lead, however, to an accumulation of a larger amount of reserve protein than the organism can with advantage retain in its fluid media. But it is entirely possible that the continuous maintenance of such an unnecessarily large supply of unorganized reserve material may sooner or later weaken one or another or all of the living tissues. At any rate it seems scarcely conceivable that the human organism, having all the time access to food, can gain in efficiency on account of such an excess of stored protein. The carrying of excessive quantities of fat is considered as an impediment, the carrying of excessive quantities

of unorganized protein may be none the less so because more common and less strikingly apparent.

Diets in diseases. — The objections that can be raised against the use of too much protein are rather meagre and indefinite with reference to perfectly normal persons, but in the case of the sick they become clear and definite. In diseases of the liver and the kidneys, in diseases affecting the formation or elimination of urea, important practical conclusions must be drawn on the basis of our understanding of the function of protein as a food constituent. If the conclusions arrived at in this paper are correct, it is clear that the exogenous protein katabolism should in such cases be reduced to the lowest practicable level. This is not accomplished by substituting a milk diet for a meat diet, for such substitution affects only the purin bodies, only the extractives. If low nitrogen experiments have any practical value, they should be tested in such cases; in other words, diets having sufficient fuel value, but containing only 3 gm. or 4 gm. of nitrogen or less should be tried. And, in special cases, as in typhoid fever or where the onset of uræmia is threatened, a diet like that used in Experiments II to V, containing practically no nitrogen, and leaving practically no undigested residue in the intestinal tract, ought to be of value. The store of nitrogen is abundant, and the use of it is lessened if non-nitrogenous food is available. Soluble starch or dextrin solution ought, it seems to me, to be more suitable than solid food, milk, or even starvation, in such cases. The use of non-nitrogenous food would seem to me to be contra-indicated only in cases where the urea elimination is enormously increased and out of proportion to the caloric needs of the body. In such cases there has probably been an extensive destruction of living protoplasm which has led to such an increase of the dissolved protein that the organism cannot retain it. While this condition lasts there may be no need of supplying non-nitrogenous food. Weakened kidneys and the elimination of albumin may perhaps, in some cases, be directly the result of an abnormal production of dissolved protein rather than the result of toxins.

The effect of work on protein metabolism. — The effect of physical work on protein metabolism, or rather the absence of any such demonstrable effect, becomes, it seems to me, for the first time intelligible in the light of the theory concerning protein metabolism advanced in this paper.

The fact that protein metabolism is independent of muscular work

seemed at first so contrary to Liebig's theory of the source of muscular energy, a theory which he defended as long as he lived, and seems still so contrary to the deep rooted general idea that meat is the most efficient kind of food for all who labor physically, that it was accepted only after having been repeatedly demonstrated by a number of different investigators. Even yet this fact has not become common knowledge. The rank and file of the people, especially the people of England and the United States, still hold the view that an abundance of meat is essential to a good day's work.

The fact that moderate, or even severe muscular work does not increase the katabolism of protein is indeed highly remarkable, if the prevailing views concerning the nature of that katabolism are correct. If the katabolism of protein consisted essentially of oxidations, and if these oxidations occurred most extensively in tissues where the greatest amount of energy is developed, *i. e.*, in the muscles, then it would seem almost incomprehensible that the tremendous *increase* of oxidation, and of energy production which accompanies muscular work, should not include an increase in the oxidation of protein. If the organism oxidizes food protein for the purpose of producing heat and energy just as it oxidizes fats and carbohydrates for that purpose, and if it in addition uses protein by preference, we should expect that the increased energy production of physical labor should increase the katabolism of protein even more than that of the non-nitrogenous food.

According to the theory developed in this paper, on the other hand, the protein katabolism, in so far as its nitrogen is concerned, is independent of the oxidations that give rise to heat or to the energy that is converted into work. The hydrolytic splitting off of ammonia, and its elimination as urea, is independent of oxidations except, of course, to the mere extent of using a relatively small amount of the carbon-dioxide that is produced by means of oxidation. According to this theory, it would therefore appear clear why work does not appreciably affect the katabolism, or, to be more specific, the exogenous katabolism of protein.

Moderately severe physical labor may, however, have more or less of an effect on the endogenous metabolism. Whether or no this is the case cannot be shown by means of urea, or even by total nitrogen determinations, because the effect of work on these constituents is too slight; determinations of kreatinin, uric acid, and neutral sulphur are necessary for the study of this question.

MEASUREMENT OF ELECTRICAL CONDUCTIVITY FOR CLINICAL PURPOSES.

By T. M. WILSON.

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A CONSIDERABLE amount of work by Stewart,[1] Tangl and Bugarsky,[2] Roth,[3] Rollett,[4] Hamburger,[5] and Viola,[6] has been done on the electrical conductivity of the blood and serum of lower animals, a physical quantity which gives us a measure of the concentration of ions in serum. The number of observations on human serum is very small, owing to the difficulty of obtaining sufficient quantities. In order to overcome this obstacle, I have made a small apparatus which requires only four or five drops of blood for each experiment, and is thus adapted for clinical purposes. It also may be of use in obtaining physiological and pathological data in animals when only small quantities of blood or other liquid are available. When the conductivity of the blood is measured, in addition to that of the serum, an observation requiring only a few minutes more, the relative volume of corpuscles and serum can be at the same time deduced either from Stewart's formula (2) "$p = \frac{\lambda_b}{\lambda_s}(174.5 - \lambda_b)$ or the more complex one $p = \frac{\lambda_b}{\lambda_s}(180 - \lambda_b - \sqrt{\lambda_b})$ formula (1)," where p is the percentage volume of serum, λ_b and λ_s, the conductivity of the blood and serum respectively, expressed in reciprocal ohms at 5° C. × 10^8.

Of course the hæmatocrite gives us an easy, rapid, and when undiluted blood is used, but not otherwise, a fairly accurate means of determining the relative volume. The additional information derived

[1] Stewart: Journal of physiology, 1899, xxiv, p. 356.

[2] Tangl and Bugarsky: Centralblatt für Physiologie, 1897, p. 332.

[3] Roth: *Ibid.*, p. 271.

[4] Rollett: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 199.

[5] Hamburger: Osmotische Druck und Ionenlehre, 1902, i, p. 474.

[6] Viola: Revista veneta di scienze mediche, xviii, Fasc. viii, 1901, *cit.* Hamburger, p. 486.

from the conductivity readings as to the approximate number of ions present in unit volume of the serum, may in the future be of equal or even greater clinical importance in certain diseased conditions.

Comparison of Electrical and Hæmatocrite Methods of Determining the Relative Volume of Corpuscles and Serum.

The electrical method of measuring the relative volume of corpuscles and serum was compared by Dr. Stewart with Hoppe-Seyler's [1] chemical method and also with a colorimetric method of his own. A satisfactory degree of agreement was found for dog's blood, for which the formulæ were originally constructed. Recently P. Fraenkel [2] has compared the method with Bleibtreu's [3] chemical method, with a like result. He recommends it for scientific purposes, but his statement that one determination can be carried out in an hour with 20 c.c. of blood and even with 12 c.c. to 15 c.c., when the blood is rich in serum, is far too unfavorable to the method, as will appear from my own results. Such quantities, of course, cannot in general be obtained in clinical examinations.

So far as I am aware, the electrical method has not hitherto been systematically compared with determinations by the hæmatocrite. Hamburger,[4] indeed, gives a table comparing the percentage-volume of corpuscles as determined by centrifugalization of a few specimens of blood with that deduced by the electrical method. But he appears to have used an ordinary centrifuge and there is considerable variation in the degree of agreement of the methods in different experiments. It was, therefore, thought worth while to begin by instituting a comparison for dog's blood between the hæmatocrite readings and the electrical determinations.

About 500 c.c. of fresh defibrinated blood from a dog was centrifugalized and the serum removed. The percentage of serum still left in the sediment as determined by the hæmatocrite was 25.5. In all the observations the hæmatocrite was turned for five minutes at an average rate of 217 times a second. The serum separated from the blood was recentrifugalized until it was entirely free from erythrocytes. Fourteen mixtures

[1] Hoppe-Seyler: Handbuch der physiologisch- und pathologisch-chemischen Analyse, 1893, p. 441.

[2] Fraenkel: Zeitschrift für klinische Medizin, lii, p. 476.

[3] Bleibtreu: Archiv für die gesammte Physiologie, li, liv, lv, and lx.

[4] Hamburger: *Loc. cit.*, p. 528.

of the sediment, with various quantities of the clear serum, were made as shown in Table I, the amount of serum added to the sediment being 5 per cent of the first mixture (Experiment 2), 10 per cent of the second mixture (Experiment 3), and so on, increasing by 5 per cent in each successive mixture. The resistance measurements were made at about 0° C., the tube being immersed in melting ice. A thermometer with its bulb close to the tube indicated a temperature which only varied one or two-tenths of a degree from 0° C. The resistances were reduced to 5° C. by multiplying by a constant. The resistance capacity of the tube was determined by filling it with 5 per cent potassium chloride solution, and measuring its resistance at 18° C., for which temperature the specific conductivity of potassium chloride solution is given in Kohlrausch's tables. From these data it is easy to calculate the conductivities of the various blood-mixtures expressed in reciprocal ohms multiplied by 10^8 at 5° C., by multiplying the reciprocals of the corresponding resistances by a constant 208,607, which is the same for all measurements made with one and the same tube. If a large series of observations is to be made, it saves time to construct once for all a table giving conductivities directly, as recommended by Dr. Stewart, and then the working out of the result from the formula entails but little labor. The small apparatus was not used for these comparative experiments, but, instead, a U-tube with a capacity of 7 c.c. A constant quantity, 5 c.c., of the mixture to be tested, was always introduced into the tube.

It will be seen that for mixtures with 67 to 74 per cent of serum, as determined by the hæmatocrite, there is a very close agreement between the results of the hæmatocrite and the electrical method. With mixtures richer in serum, the electrical method gives results somewhat higher, and with mixtures poorer in serum, results somewhat lower than the hæmatocrite. At both extremes the numbers deduced from formula (1) are nearer to the hæmatocrite readings than those deduced from formula (2). But in the middle range, which would correspond to the majority of specimens of dog's blood, formula (2) gives a somewhat closer approximation to the hæmatocrite determinations. As in Dr. Stewart's[1] tables when the percentage of serum is low, formula (2) gives rather smaller values than formula (1), but somewhat larger values when the percentage of serum is high. Some serum is, of course, present in the interstices of the sediment in the hæmatocrite tube even after prolonged centrifugalization. No attempt was made to determine its amount. But the

[1] Stewart: *Loc. cit.*

TABLE I.

No. of exp.	Sediment taken in c.c.	Serum added in c.c.	Conductivity.	Percentage of serum by electrical method.		Serum readings by hæmatocrite.	Hæmatocrite readings corrected for serum still between the corpuscles, assuming that it is		Percentage of serum in the mixtures, calculated on assumption that hæmatocrite sediment contains of serum		
				Formula (1).	Formula (2).		5 per cent.	10 per cent.	None.	5 per cent.	10 per cent.
1	5.00	0.00	12.30	24.3	24 0	25.5	29.2	32 95	25.5	29.2	32.95
2	9 50	0.50	14 05	27 6	27.3	29.6	33.1	36.60	29.2	32.7	36.30
3	4.50	0.50	16.30	31.5	31 2	34.0	37.3	40 60	32.9	36.3	39.60
4	5 66	1 00	19.70	37.3	36.9	38.5	41.6	44 60	36 6	39.8	43.00
5	4 00	1 00	21 80	40.5	40.3	42 0	44.9	47 80	40.4	43.3	46.30
6	4 00	1.50	24.00	43 8	43 6	46.1	49 1	51.70	44.1	46.9	49.60
7	3.50	1.50	26.50	47 5	47.4	50.5	53 0	55.40	47.8	50.4	53.00
8	3 25	1.75	30.30	53 0	53.0	56.6	58.7	60 90	51 5	54.3	57.10
9	3.00	2.00	33.90	57.8	57.9	60.0	62.0	64.00	55.3	57.5	59.70
10	3.66	3.00	36 60	61.0	61.2	63.0	64 8	66 70	59.0	61.0	63.10
11	2.50	2.50	41.10	66.2	66.7	67.0	68 6	70.30	62.7	64.5	66.40
12	3.00	3.33	44.90	69.8	70.5	70.0	71.5	73 00	66.2	67.2	68.20
13	2.00	3.00	48.60	73 5	74.3	74.0	75 3	76.60	70.2	71.7	73.20
14	3.50	6.50	53.10	77.2	78 3	76 3	77 5	78.60	73.9	75.2	76 50
15	1.50	3.50	56.80	77.9	81.1	80.0	81.0	82 00	77.6	78.8	79.90

The conductivity of the serum was 82.4.

magnitude of the errors which may possibly arise, neglecting this quantity, are displayed in the table, where the percentages of serum actually present in the mixtures are calculated on the assumptions: (a) that no serum was still present between the corpuscles in the hæmatocrite sediment, (b) that 5 per cent of the hæmatocrite sediment consisted of serum, and (c) that 10 per cent of it was serum. The actual hæmatocrite readings are given, and also readings corrected in accordance with assumptions (a), (b), and (c). The computed percentages are uniformly lower than the corresponding hæmatocrite readings, which seems to indicate that there may be some truth in the criticism directed against the hæmatocrite method that, even when undiluted blood is employed, liquid may be squeezed out of the corpuscles during centrifugalization.

The common method of diluting blood with artificial solutions before centrifugalization is pretty generally recognized as inadmissible when any great degree of accuracy is required, since, in general, the diluting solution will not be exactly isotonic with any particular specimen of blood.

The calculated percentages agree considerably better with the electrical determinations than with the corresponding hæmatocrite readings for mixtures relatively rich in serum, and for those containing such a proportion of serum as is generally found in normal blood.

For mixtures relatively poor in serum the computed percentages diverge considerably from the actual hæmatocrite readings, though much less from the corrected readings. The electrical results for mixtures poor in serum agree fairly with the percentages calculated on the assumption that the serum in the interstices of the hæmatocrite sediment is negligible in amount, but diverge rather widely for percentages calculated on assumptions (b) and (c).

Description and Testing of Apparatus for Measuring Conductivity of Human Blood and Serum.

The small apparatus for human blood is shown in Figure 1.

Its length is 50 mm. so as to be adapted to the ordinary hæmatocrite frame. It is 6 mm. in outer diameter and 3 mm. bore. The ends are ground on an emery wheel and made to fit into the sockets of the frame. More than half of the length of the tube is plugged with a glass rod and sealed tightly with sealing wax. The capacity of the part remaining, that is from *C* to *A*, is 0.1322 c.c., as determined by weighing the tube filled with dis-

tilled water and then weighing it empty. The platinum electrodes are fixed in a glass piece which can be inserted in the liquid filling the tube.

In Fig. 2, *G* is the glass electrode head surrounding the platinum wires, *W*, which are about 0.5 mm. in diameter. These wires are bare at *P* and

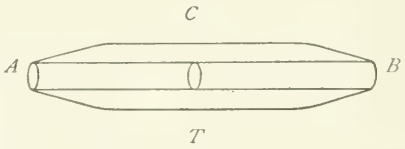


FIGURE 1. — *T* = tube; *A* = open end of tube; *B* = closed end; *C* = upper end of glass plug.

N. The whole figure represents a section of the electrodes. The part from *F* to *P* is sufficiently small to enter the bore of the tube T (Fig. 1) without coming in contact with the sides. *N* and *P* in Fig. 2 are the only parts of the electrodes which are not insulated with glass.

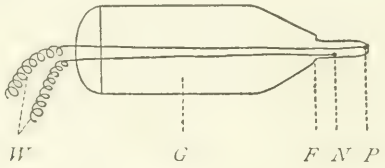


FIGURE 2. — *W* = wires; *G* = glass electrode head; *F* = flange of glass; *N* = upper electrode; *P* = lower electrode.

Figure 3 is a section of the complete apparatus. *C* is an aluminium cap which is firmly sealed to the electrode head *H*. The cap is so bevelled internally as to resemble two truncated cones with apices adjoining. The tube *T* can be thrust into one of these conical spaces as is seen in the figure. When thrust home, the tube *T* fits into the cap in a definite position such that the insulated portion of the electrode head which enters

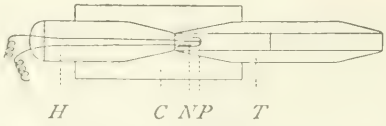


FIGURE 3. — *H* = glass electrode head; *C* = aluminium cap; *N* = upper electrode; *P* = lower electrode; *T* = tube.

the bore does not touch the sides of the tube, so that when the tube is filled with blood or other fluid, a layer of it intervenes between the electrodes and the tube. When in position, electrode *N* is 2 mm., and electrode *P* 10.5 mm. from the open end *A* (Fig. 1).

Figure 4 shows the apparatus in a vertical position. The tube T fits so tightly into the cap *C* that it is held suspended. Its constancy of position is assured by making the marks *M* and *M'* on the cap and tube coincide. The whole apparatus, after the tube is filled with blood, is suspended in a narrow metal tube which is surrounded with pounded ice. In the bottom of the aluminium tube was placed some mercury to aid in more rapid cooling. The apparatus was pushed so far down into the metal tube that the top of it was 4 cm. below the surface of the ice.

The electrodes were covered with platinum black in the usual way, by means of a galvanic current passed through a 2 per cent solution of platinic chloride.

A medicine-dropper drawn to a fine point was used to fill the tube *T*, and afterwards to wash its bore and head with water. A pair of bellows was employed to thoroughly dry the whole apparatus after each experiment.

The first test made with the apparatus was to compare its readings with those obtained with the U tube employed in the experiments shown in Table I.

Two samples of dog's blood A and B were used for these tests. The U tube and the small apparatus were filled at the same time and placed in position in the same vessel containing pounded ice, and readings were made after six minutes' immersion. Table II shows the values obtained. For sample A, the ratio of the average of all the readings with the U tube to the average of all the readings with the small apparatus was 0.5108, and for sample B, the corresponding ratio was 0.502, so that the agreement is satisfactory.

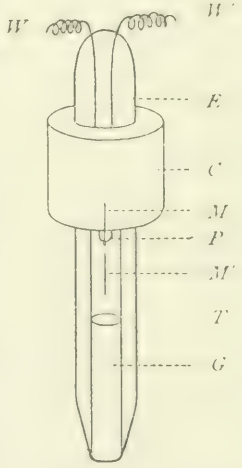


FIGURE 4. — *E* = glass electrode head; *C* = aluminium cap; *M'M* = adjusting lines; *T* = tube; *G* = glass plug.

As it is convenient to defibrinate the blood before filling the small apparatus, especially for clinical work, the question arises whether defibrination causes any difference in the conductivity of the serum. G. N. Stewart[1] found that the conductivity of the serum of clotted blood is approximately the same as that of the serum of defibrinated blood from the same animal. Oker-Blom[2] states that the serum

[1] STEWART: Centralblatt für Physiologie, 1897, p. 332.

[2] OKER-BLOM: Osmotischer Druck. HAMBURGER: 1902, p. 477.

separated from the blood clot has a greater conductivity than the serum obtained from defibrinated blood; Hamburger[1] asserts that the

TABLE II.

Blood A.		Blood B.	
Small apparatus.	U tube.	Small apparatus.	U tube.
8,000	4,090	8.010	4.010
7,900	4,090	7.900	4.000
8,000	4,090	7.985	4.005
8,000	4,090	8.000	4.000
8,100	4,080		
8,000	4,090		
8,000	4,080		
Average 8,000	4,087	Average 7,973	4.003

freezing point of the serum of coagulated blood is lower than that of the serum from defibrinated blood. I made the following experiments in the hope of clearing the matter up.

A dog was anæsthetised by ether. Blood was removed by inserting a cannula in the carotid, and one portion was allowed to clot and the other defibrinated. Both samples were placed in the refrigerator for six hours. The serum of each was then obtained. Both sera were slightly tinged with blood-pigment, but the tint in the serum from the defibrinated blood was a little deeper. The coloring matter proved, on standing, to be still in the corpuscles in both sera. The deeper tint in the defibrinated serum was due to it having been left for a shorter period in the centrifuge. A colorimetric comparison showed that the defibrinated serum had 0.1 per cent of corpuscles and the serum from the clot 0.095 per cent. As can easily be calculated, the difference in conductivity which could arise from this difference in the content of corpuscle is quite negligible, since 1 per cent of corpuscles would only alter the conductivity about 1 by 10^8 in the unit in which conductivity is expressed in the paper.

[1] Hamburger: *Ibid.*, p. 477.

Four samples of each serum were taken and the conductivity determined by means of the U tube. Freezing-point readings were also made. The results are given in Table III. They indicate that the

TABLE III.

	Clot Serum.		Defibrinated Serum.	
	$\lambda \times 10^{-8}$.	Δ.	$\lambda \times 10^{-8}$.	Δ.
1	87.2	0.668	87.2	0.660
2	87.2	0.665	87.6	0.670
3	87.6	0.660	87.2	0.665
4	87.2	0.660	87.2	0.660

conductivity and freezing-point of the serum are the same whether it is derived from the clot or from the defibrinated blood.

In close connection with the above may be mentioned an experiment with the small apparatus, on blood obtained from a healthy student, to ascertain if there was any difference in the conductivity of

TABLE IV.

No. of exp.	Ratio of resistance of serum and blood.	Constant cor. for 0° C. giving values in 5° C.	Conductivity of blood.	Percentage of serum by formula (2).	Percentage by hæmatocrite.
1	3860 : 8360	395890	47.4	58.5	58.0
2	3870 : 8300	"	47.6	59.1	57.4
3	3860 : 8400	"	47.1	58.2	58.3
4	3870 : 8830	"	44.8	56.8	57.9
5	3840 : 8700	"	45.5	56.7	58.0
6	3840 : 8500	"	46.5	57.8	58.0
7	3840 : 8570	"	46.1	57.5	58.0
	Average		46.4	57.8	57.9

defibrinated and non-defibrinated blood, and between the conductivity of plasma and serum from defibrinated blood. The conductivity for

unclotted blood was 37.5; for defibrinated blood, 37.7; for serum from defibrinated blood, 91.2; for plasma, 91.6.

These results would indicate that the conductivities of defibrinated blood, or unclotted blood, and of plasma, or defibrinated serum, may be taken at will without introducing any appreciable error in our calculations.

Conductivity and hæmatocrite determinations were now made with the small apparatus on dog's blood, the same precautions being observed as with the U tube. The results (in Table IV) again show a fairly satisfactory agreement. There is, however, a greater variation in the individual electrical determinations than would now be considered justifiable after the apparatus and technique have been improved in various ways.

Experiments with the Small Apparatus on Human Blood.

Blood from healthy students was obtained by puncturing the volar surface of the thumb with a sharp scalpel. The incision was about

TABLE V.

Student's number.	Conductivities of blood at 5° C. × 10^3.	Conductivities of the plasma at 5° C. × 10^8.	Percentage of plasma by formula (2).
1	35.3	101.1	48.1
2	52.6	107.0	52.2
3	48.2	111.0	52.9
4	41.1	95.0	59.5
5	51.7	101.0	62.3
6	39.0	84.0	62.3
7	53.8	92.9	55.2
8	48.1	95.8	62.5
9	36.5	89.0	57.6
10	37.9	89.5	57.8
11	46.5	109.0	54.3
12	49.6	102.6	56.8
13	50.1	109.2	56.9

2 mm. deep, and five drops of blood were caught in a crucible resting on powdered ice. The tube was filled at once without defibrinating the blood and adjusted to the electrodes. The apparatus was inserted six minutes in the outer metal tube, surrounded with powdered ice, before the readings were taken. The results are given in Table V.

The above results give a range in conductivity of serum from 84 to 111, of blood from 35.3 to 53.8, of percentage of corpuscles from 48.1 to 62.3.

Viola[1] gives a range for the conductivity of serum of healthy individuals from 106.18 to 119.12 (expressed in reciprocal Siemen's units at 25° C.) The series was obtained from the blood of eight individuals. Converting these values into reciprocal ohms at 5°C., we get a range of from 82.6 to 92.7, which is considerably narrower than mine.

Table VI gives results obtained on a healthy man when food and drink were taken. The blood was defibrinated by means of a needle. An interval of seven minutes was always allowed before making a reading, as there was some question whether the six minutes allowed in the preceding series was sufficient to permit the blood in the tube to cool completely down to 0° C. A shorter time did not seem to give quite constant readings. Doubtless the use of a still narrower outer metal tube would lessen the time required. The range of the serum conductivities in this individual over a period of more than five weeks, when observations were made under conditions calculated to bring out the influence of the ingestion of food and water, was from 86.5 to 111, which is very nearly the same as the range for single observations on the series of healthy students in Table V. The range of conductivities for the blood in Table VI was much narrower. In general a pretty constant inverse ratio is seen between the percentages of the serum and its conductivity, that is to say, when the percentage of the serum is high its conductivity is relatively low and *vice versa*. It is worthy of remark that the results in Experiment 12 of this table, in which a pathological condition (diarrhœa) was present, vary most from this inverse ratio. This is well illustrated by the last column in Table VI. which gives the products of the percentages of serum into the corresponding conductivities. It will be seen that there is a much closer approximation between the products than between the conductivities or percentages. The meaning of this would seem to be, that when the relative volume of serum is increased, *e. g.*, by

[1] Viola: Hamburger: *Loc. cit.*, p. 484.

drinking water, the serum becomes more dilute as regards salts, and therefore has a diminished specific conductivity. When the serum diminishes in amount, water seems to pass out of it in greater propor-

TABLE VI.

No. of exp.	Date of experiment.	Conditions of experiment.	Conductivity of blood at 5° C.	Conductivity of serum at 5° C.	Percentage by formula (2).	Percentage by hæmatocrite.	Percentages of serum multiplied by the conductivities of serum.
1	Oct. 10	1 hr. after meal	45.0	106.8	54.6	54.0	5831
2	Oct. 11	4 hr. after meal	40.2	86.6	62.4	54.0	5403
3	Oct. 25	Just after dinner	46.7	111.0	53.7	51.5	5960
4	Oct. 26	1 hr. after dinner	43.6	98.4	57.5	52.0	5658
5	Nov. 22	3 hr. after meal	45.0	104.0	56.1	52.0	5834
6	Nov. 22	Drank 250 c.c. distilled water; waited 30 min.	43.2	96.0	59.0	52.0	5664
7	Oct. 26	1 hr. after dinner	43.4	98.4	57.5		5592
8	Oct. 26	Drank 500 c.c. distilled water; waited 30 min.	43.4	94.6	60.2		5640
9	Nov. 5		42.9	93.5	59.1		5491
10	Nov. 5	Drank 250 c.c. distilled water; waited 1 hr.	41.0	89.0	61.5		5433
11	Nov. 8		41.5	110.0	50.2		5500
12	Nov. 8	Drank 500 c.c. distilled water; waited 1 hr.	41.0	95.8	58.3		5517
13	Nov. 9	Mild diarrhœa	46.1	99.2	60.9		6041
14	Nov. 16		41.0	94.9	57.8		5485
15	Nov. 16	Drank 250 c.c. distilled water; waited 20 min.	40.7	92.9	58.8		5462
16	Nov. 17		42.2	95.8	58.4		5594
17	Nov. 17	Drank 250 c.c. distilled water; waited 30 min.	41.5	93.5	59.1		5525

tion than salts. The conductivity of blood varied very slightly, only 5.7, which is much less than the variation for healthy individuals in Table V. The first five experiments also give the percentages of serum obtained by the hæmatocrite, and differ from 0.6 to 8.4 per

cent, the hæmatocrite readings being invariably lower. I am unable to suggest the reason why, in the observations in Table VI, in which the hæmatocrite readings were taken, these readings are invariably lower than the percentages of serum obtained by the electrical method, whereas, in the observations on dog's blood (Table I), the hæmatocrite readings for corresponding percentages of serum were a little higher than the electrical determinations. It may be that the centrifugalization was not so complete in the observations in Table VI, as the hæmatocrite was not turned so long. But as I always centrifugalized till two successive readings showed no appreciable difference, probably this is not the correct explanation. Whether a greater proportion of serum may not remain in the sediment of human blood than in that of dogs, I am unable to state. This is possible, but perhaps not likely. Finally it may be that the formula deduced from conductivity observations on dog's blood is not quite accurate for human blood. Fraenkel[1] indeed, states that he obtained a close agreement between Bleibtreu's method and the electrical method (using Stewart's formula) for human blood. But he only used one specimen of healthy human blood. I am unable here, as in Table V, to compare my results with those of other investigators, since I find no published results on this subject.

Table VII gives a few results on clinical cases. Hæmatocrite readings were obtained by using the resistance tube as a hæmatocrite

TABLE VII.

Number of case.	Conductivity of blood at 5° C.	Conductivity of serum at 5° C.	Percentage of corpuscles by formula (2).	Percentage of serum using tube T as hæmatocrite.
1	45.2	96.6	60.5	53.0
2 A	82.6	93.0	81.3	90.4
B	81.5	92.4	82.0	90.0
3	64.3	105.6	68.8	62.0
4	44.0	97.6	58.8	50.9
5	37.7	91.6	56.2	52.8

tube, the length of the columns of corpuscles and serum being measured by means of a pair of calipers and by the aid of a hand lens.

[1] FRAENKEL: *Loc. cit.*

These readings differed by less than 2 per cent from those obtained by the regular hæmatocrite tube, and of course by etching a scale on the tube, still greater accuracy could be obtained. There is, moreover, the advantage that the tube is more easily filled and cannot possibly leak, as so often happens with the ordinary tube.

Case 1. — Blood from a boy, twelve years of age, with adenoids; temperature 101°.

Case 2. — Determinations were made on two specimens of blood (A and B) taken from the heart of a man four hours after death from pernicious anæmia. Clotting did not take place until two and one-half hours after removal. The blood count just before death was 816000 reds and 2900 whites, hæmoglobin, 18 per cent.

Case 3. — Blood obtained from a woman two days after labor.

Case 4. — Blood from a woman in the last stages of Bright's disease.

Case 5. — Patient was suffering from acute gastritis, and had been given four drachms of Epsom salts and two grains of calomel within the previous four hours, but without being purged.

In the above five cases the blood was defibrinated, hermetically sealed in a fine tube and thus conveyed to the laboratory.

Table VII shows a greater variation in the conductivity of blood than that found in normal individuals, the range being from 37.7 to 82.6. There was no corresponding variation in the conductivity of the serum. Viola[1] gives a range for the conductivity of serum in blood obtained from a few post-mortem cases of 98 to 140 expressed in reciprocal Siemen's units at 25° C. or 76.3 to 109 in reciprocal ohms at 5° C.

Summary.

1. The electrical method of determining the relative volume of corpuscles and plasma gives results (in the case of dog's blood) which upon the whole agree fairly well with the hæmatocrite determinations.

2. The conductivity of serum from clotted blood is within the limits of error, the same as that of the corresponding plasma and of the corresponding serum from defibrinated blood. The same is true of the freezing-point of serum from clotted blood and from the corresponding defibrinated blood.

3. The small apparatus described enables us to determine within less than 1 per cent the electrical conductivity of serum and less

[1] Viola: Hamburger: *Loc. cit.*, p. 484.

than 2 per cent the conductivity of blood obtained in such quantities as are easily available for clinical examinations (four to five drops of blood). The time necessary for the determination of the conductivity of serum (including the defibrination and centrifugalization of the blood) is from ten to thirteen minutes, of which about seven minutes are required for the conductivity measurement; seven minutes more, making twenty minutes in all, are required for obtaining the conductivity of the blood, and thus for determining the relative volume of corpuscles and plasma. The resistance tube can be used as a hæmatocrite tube.

4. In thirteen healthy students the conductivity of the serum varied from 84–111, and that of the blood from 35.3 to 52.6.

5. In one and the same healthy individual the conductivity of the serum, determined at intervals over a period of more than five weeks, and at various times after taking food and water, varied from 86.5–111, and that of the blood from 40.2 to 46.7.

6. In a few cases of disease the conductivity of the serum varied from 91.6 to 105.6, while that of the blood varied from 37.7 to 82.6 the latter in a case of pernicious anæmia.

In conclusion, I have to thank Professor G. N. Stewart for his many kindnesses in suggesting this investigation, and in assisting me with it.

THE EFFECT OF SALT-SOLUTIONS ON CILIARY ACTIVITY.

By S. S. MAXWELL.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE most extensive studies upon the effect of inorganic salts on cilia have been made by Lillie[1] and by Weinland.[2] Lillie investigated the action of certain chlorides and mixtures of chlorides upon the muscular and ciliary activity of the larvæ of Arenicola and Polygordius. Both these forms are marine and the cilia are exposed normally to sea-water with its high content of inorganic salts. Most of the parallel studies upon developing eggs and embryos have been made upon marine forms. It would seem desirable that similar studies should be carried on at least as extensively upon tissues of fresh water or terrestrial animals before too sweeping generalizations are formulated. Weinland's experiments were made on the ciliated epithelium of the frog's mouth and œsophagus; but in order to study the stimulating action he used strong solutions, $\frac{1}{2}$ mol. in most instances, and for this reason his results do not at all correspond to the effect of solutions of the same substances in strengths more nearly approximating the osmotic pressure of the cells. The experiments described in this paper were undertaken for the purpose of investigating the effects of pure solutions of single salts upon the ciliated epithelium of the frog's mouth and œsophagus in order to discover what general relation, if any, exists between the properties of the salts and their physiological action upon the tissue.

In order to simplify the problem a limited series of salts having a common anion was employed; namely, the chlorides of lithium, ammonium, sodium, potassium, magnesium, calcium, strontium, and barium. These were employed in $\frac{1}{8}$ mol. solutions. The salts used were the purest obtainable. Most of them were recrystallized, some of them several times. It was found to be especially important to avoid any trace of acid or alkali, since even a minute quantity has

[1] LILLIE: This journal, 1901, v, p. 56; 1902, vii, p. 25; 1904, x, p. 419.

[2] WEINLAND: Archiv für die gesammte Physiologie, 1894, lviii, p. 105.

a marked influence on the life of the cilia. The solutions were made up from water distilled in glass. All the solutions were prepared by exactly similar methods, and contained, presumably, fairly equal quantities of dissolved oxygen.

The action of these solutions was investigated by two methods: 1. The effect which more or less prolonged immersion of the epithelium in the solution produces upon the work of the cilia. 2. The duration of life of the ciliated cells while immersed in the solutions.

Preliminary Experiments on Work of Cilia.

The pioneer investigations on the work of the cilia were made by Wyman,[1] Engelmann,[2] and Bowditch.[3] The method employed by Bowditch was the only one which gave results in measurable terms of mechanical work. He placed the epithelium upon an inclined plane of known gradient so that the cilia by their action moved a load of known surface and weight against gravity. The movement was observed with a microscope containing a micrometer-ocular, and the rate determined with a stop-watch. From the known factors and the observed rate, the work was calculated in gram-millimetres per minute for a square centimetre of surface. In my experiments practically the same method was employed. Experience proved that the cilia were very sensitive to changes of temperature. Air-currents were especially disturbing probably on account of changes in amount of evaporation from the surface of the epithelium. For this reason the inclined plane was placed in a galvanized iron tank 46 cm. long, 21 cm. broad, and 15 cm. deep, through the glass cover of which observations were made. The tank was kept half full of water. This arrangement insured a good air-supply and a relatively constant temperature. The inclined plane used was of glass and had a gradient of 10 per cent. A rubber band around it formed a stop against which rested, during the observations, the paraffin block described below. In this way it was made certain that exactly the same portion of the preparation was used in all the observations. An eyepiece-micrometer was used one hundred divisions of which equalled a distance of $8\frac{8}{9}$ mm. and therefore a lift against gravity of eight-ninths of a millimetre. The time was measured with a stop-watch.

[1] Wyman: American naturalist, 1871, v, p. 611.

[2] Engelmann: Archiv für die gesammte Physiologie, 1877, xv, p. 493.

[3] Bowditch: Boston medical and surgical journal, 1876, xv, p. 159.

The carriages employed were circular pieces of hard rubber 8.6 mm. in diameter, and weighing, one of them, 415 mgm., the other, 500 mgm. In connection with each of these was a set of lead weights which, placed on top of the rubber carriage, increased the load by even multiples of the original weight. In the description of experiments following it will be understood that the weight of the carriage employed will be considered the unit load.

For use in these experiments the epithelium of the roof of the mouth was dissected out in connection with the œsophagus, which was cut open longitudinally on its ventral side. The preparation was spread out flat upon a paraffin block and pinned down with glass pins, care being taken to avoid wrinkles by sufficient stretching. The paraffin blocks were about 50 mm. long, 25 mm. broad, and 8 mm. thick, and were formed upon pieces of glass of the same rectangular area to weight them down when in the salt-solutions. During the time occupied in dissecting and pinning out the preparations, considerable drying occurred, and observations made without moistening were found to be somewhat variable, the amount of variability being much greater in some preparations than in others. Thus successive trials in one preparation gave the following times in seconds occupied in carrying the 415 mgm. weight over 100 divisions of the micrometer-scale: 21, 25, 22, 24, 26, 25, 30, 32, 24, 23. Another preparation gave 16, 15, 15, 14, 25, 20, 20, 20, 22, 17. The most constant results were obtained from specimens, moistened with frog-blood serum. The advantage of this is shown by the following rates from one of the preceding specimens, after moistening with serum: 17, 17, 16, 16, 17, 17, 17, 16, 17, 17. It was usually necessary, however, to reject the first few observations, because of the increasing irritability of the preparation, discussed below under the heading "Staircase phenomenon."

In all these experiments one must take into account the great individual differences between the specimens used even when the frogs have been just freshly taken, and these differences become much more pronounced when the animals have been kept for some time under the unnatural and relatively unhealthful conditions due to confinement in the laboratory tanks. For this reason it was decided to throw out of consideration as abnormal all specimens which, on the first six or eight trials, when moistened with blood-serum, required longer than fifteen seconds to move 0.5 gm. weight a distance fifty divisions of the micrometer-scale; experience with such specimens

showed that their cilia became inactive in a very much shorter time than those of normal preparations kept under identical conditions.

Effect of Load.

Before entering upon the investigation of the effects of the various salts, a study was made of the effect of load and of fatigue upon the power of doing work. These experiments were made upon preparations moistened with the ordinary laboratory physiological salt-solution, made up from commercial salt and tap-water, and also with specimens moistened with frog-blood serum. Although these experiments were merely preliminary, the results appear to have some interest in themselves, and are here reported in fuller detail than their relation to the subject would require.

Under the conditions described above, if one begins with the unit-weight of say 0.5 gm., and then uses successively two, three, four, five, etc., times this unit, until a weight is reached which the cilia do not move, it will be found that the rate of movement has declined rather steadily; but that on employing the unit-weight again, the movement is slower than at first. The effect of fatigue has lengthened the time-intervals for the heavier weights. On this account, it is necessary to use larger multiples of the unit. In practice it was found convenient to use a series of loads in the proportion of 1, 4, 7, 10, 13, 16, 19, and 22. Even with such a series the results are far from being as regular as could be desired. It is, therefore, necessary to make a very large number of observations with frequent controls.

After trying various procedures, the plan finally adopted was to make a control with the unit-load, after each experiment with a heavier load. If the control at any time varied more than one second from the initial rate, a short period of rest was allowed to intervene, and the rate with unit-load was taken again. For example Specimen H of October 29 gave:

														Rest 2 min.		Rest 4 min
Load	1	4	1	7	1	10	1	13	1	16	1	1	19	1		1
Rate in seconds	8	10	7	12	7	15	8	21	8	24	10	9	60	10		10

Whenever, as in this instance, after load 19, the rate with unit-load did not return to within one second of the initial rate, the experiment was broken off, because the figures obtained for the heavier weights would not be fairly comparable with those obtained for the lighter ones.

TABLE I.

EFFECT OF LOAD ON MECHANICAL WORK OF THE CILIA.

In each experiment the upper line gives the number of seconds required to carry the load 50 degrees of the micrometer-scale, and the lower line is the corresponding work reckoned in gram-millimetres per minute for each square centimetre of epithelium.

Relative wt. of load	1	4	7	10	13	16	19	22
Absolute wt. of load	0.415	1.66	2.905	4.15	5.395	6.64	7.885	9.13
Experiment 1	9 2.117	15 5.08	19 7.018	24 7.937	30 8.255	48 6.35	310 1.167	∞ 0
" 2	7 2.721	10 7.62	11 12.123	18 10.583	26 9.525	35 8.709	225 1.609	∞ 0
" 3	10 1.905	12 6.35	14 9.525	15 12.70	20 12.382	30 10.16	30 12.06	—
" 4	8 2.381	10 7.62	12 11.112	15 12.70	21 11.793	24 12.70	60 6.03	—
" 5	7 2.721	9 8.467	12 11.112	15 12.70	19 13.034	25 12.192	48 7.541	—
" 6	6 3.175	8 9.525	11 12.123	13 14.654	17 14.568	24 12.70	40 9.048	—
" 7	9 2.117	14 5.443	16 8.334	23 8.283	34 7.284	50 6.096	500 0.724	∞ 0
" 8	8 2.381	11 6.927	14 9.525	25 7.62	33 7.504	40 7.62	—	
" 9	10 1.905	14 5.443	21 6.35	35 5.443	33 7.504	—		
" 10	10 1.905	14 5.443	19 7.018	21 9.071	30 8.255	50 6.096	42 8.617	—
" *11	15 1.27	31 2.458	51 2.614	114 1.671	—			
" 12	15 1.27	33 2.309	57 2.338	95 2.005	—			
" 13	13 1.461	25 3.048	40 3.334	∞ 0				

* The preparation used in Experiment 10 was placed in the laboratory normal saline solution and after twenty-four hours gave the results shown in Experiment 11. Experiments 12 and 13 were also made on preparations which had been twenty-four hours in normal saline.

Table I shows the results of thirteen experiments. The loads are in the ratios mentioned above, namely, 1, 4, 7, 10, etc. The unit-weight was 0.415 gm. The weights of the loads used are given in grams in the second line of the table. In each experiment the figure above gives the time in seconds for fifty divisions of the millimetre-scale. The lower number in each space gives the work in gram-millimetres per minute for each square centimetre of epithelium. A dash in the position for seconds in the table shows that the experiment had to be ended, because the preparation did not recover from fatigue.

Table II differs from Table I only in the fact that the unit-load is 0.5 gm. The ratios of loads and the arrangement is the same as in Table I.

TABLE II.

Data arranged as in Table I.

Relative wt. of load	1	4	7	10	13	16	19	22
Absolute wt. of load	0.5	2.0	3.5	5.0	6.5	8.0	9.5	11.0
Experiment 1	8 2.868	12 7.649	17 9.449	25 9.179	33 9.04	50 7.342	200 2.18	∞ 0
" 2	7 3.278	11 8.345	18 8.922	22 10.40	30 9.944	42 8.742	70 6.229	∞ 0
" 3	8 2.868	13 7.061	21 10.928	30 9.944	—			

It will be seen from inspection of these tables that the curve of work of the cilia rises at first rather rapidly with increasing load, maintains itself near the maximum through a considerable range, and then falls again rapidly to the point where the load cannot be moved at all.

The maximal in most cases is higher than that obtained by Bowditch, 6.805 gram-millimetres per square centimetre. The difference is probably due to the different size of carriage employed, 1.437 square centimetres in his experiments, and 0.581 in mine; but this has not been sufficiently investigated. The shape of the carriage, oval in the one case and circular in the other, may also be a factor.

It is evident, of course, that the figures given above represent only a fraction of the actual work of the cilia, for the force of their strokes

is applied, not directly to the load to be moved, but to the liquid with which its surface is moistened. While this is true, it seems fair to assume that the degree of regularity shown indicates that actual relations are exhibited, although not actual quantities. The quantity and quality of mucus or other liquid present very probably affects the results; but this did not prove so important a factor as might be expected. Except where a clotted mass of material was present, very little difference was observed in the rates before and after removal of so much of the mucus as could be taken off with a moist camel's-hair brush. It is possible that the three cases given in Table I, Experiments 11, 12, and 13, where the preparation had been for twenty-four hours in a normal saline solution, may indicate the necessity of mucus in the moving of heavy loads. In each of these, the curve of work falls very rapidly. These preparations, owing to the exhaustion of the goblet cells, are almost free from mucus, and the slow rate of the heavy weights might be due to the lesser adhesiveness of the liquid between the carriage and the epithelium. On the other hand, such preparations are especially susceptible to fatigue, from which they recover with extreme slowness, and this would suggest that the real cause of the characteristic form of their work-curve is an actual lowering of their working power, and not a change in the character of the liquid with which they are moistened.

Staircase-Phenomenon.

Kraft[1] and others have called attention to the fact that cilia are markedly responsive to mechanical stimulation. There is also good reason for believing that up to a certain degree each stimulus causes the cilia to become more irritable to succeeding stimuli. I find among my notes many records, in which, at the beginning of the experiment, the rate has increased with a number of successive applications of the same load. In some of them the staircase-effect is quite as well marked as in the characteristic examples of muscular work. For example, in an experiment of October 13, the following successive rates were obtained : 28, 23, 17, 16, 15, 13, 14, 12, 13, 13 In another experiment, October 10, the rates were 27, 26, 22, 23, 18, 17, 17, 17. In other instances, the first trial or two are markedly slower than the succeeding ones, which then run along with a fair degree of uniformity.

[1] Kraft: Archiv für die gesammte Physiologie, 1890, xlvii, p. 215.

A similar phenomenon was often seen in specimens which had been kept for some time in salt-solution, for example, a preparation which had been twenty-seven and a half hours in normal saline solution gave the following: 30, 15, 14, 13, 13, 13, 13. It is possible that in these cases the solution was deficient in oxygen, and that the increase in rate was due to gradual recovery from partial asphyxiation; but the phenomenon is so similar to that seen in fresh specimens, that such an assumption is unnecessary.

Fatigue.

The occurrence of fatigue of the cilia has been already mentioned in discussing the effect of work. It might be supposed that the slowing of rate, which occurs after heavy loads have been moved, is not a true fatigue phenomenon, but is caused by accumulation of mucus, or by drying. That this is not true is shown by the fact that often a few minutes' rest is sufficient, without removal of mucus or addition of moistening liquid, to restore the original rate; and also by the fact, already mentioned, that preparations which have stood for a considerable time in normal saline solution are much more quickly fatigued than fresh specimens. In such specimens, the goblet cells are practically exhausted, and the surface is almost free from mucus.

The Effect of Salt-Solutions upon Working Power.

Table III shows the results of the experiments with the eight solutions. Each preparation was moistened first with frog-blood serum, and the rate determined with the 0.5-gm. load. It was then washed with the solution and the rate determined again; a third determination was made after one hour's immersion in the solution, a fourth after four hours, and, if still active at that time, the preparation was tested again at the end of twenty-four hours. The figures given are the seconds required to move the 0.5-gm. weight fifty divisions of the micrometer-scale. Where a cross appears in the column, it means that the weight moved perceptibly but so slowly that the determination of the exact time would have had no special significance. A zero indicates that the weight did not move. Since all the figures are for the same load, it is unnecessary to present the calculation of work done. The work is, of course, in inverse proportion to the rate. In these observations, the rate for the 2-gm. and the 5-gm. loads was also recorded, but no fact of importance was brought out by them.

TABLE III.

Effect of salt-solutions on working power of the cilia. Seconds required to move 0.5 gm. fifty divisions of the micrometer-scale.

Salt.	No. of exp.	In serum.	In solution.	1 hour.	4 hours.	24 hours.
LiCl	1	7	10	+	0	
	2	9	14	126	0	
	3	11	14	0	0	
	4	14	16	+	0	
NH_4Cl	1	11	6	50	+	0
	2	9	6	60	0	
	3	13	9	90	0	
	4	12	11	180	0	
NaCl	1	9	9	17	17	0
	2	13	7	14	15	0
	3	13	9	20	22	+
	4	7	7	21	23	+
KCl	1	11	7	85	+	0
	2	8	9	62	0	
	3	15	7	170	+	0
	4	14	11	+	0	
$MgCl_2$	1	10	10	43	0	
	2	8	9	23	0	
	3	11	11	24	0	
	4	13	11	74	0	
$CaCl_2$	1	9	7	35	+	0
	2	8	8	87	0	0
	3	12	9	84	0	0
	4	13	11	72	0	
$SrCl_2$	1	12	7	26	0	
	2	15	12	30	0	
	3	9	7	15	420	0
	4	9	10	20	740	0
$BaCl_2$	1	8	10	+	0	
	2	15	10	+	0	
	3	10	7	0	0	
	4	9	8	+	0	

+ The weight moved perceptively, but so slowly that the determination of the exact time would have had no special significance.

0 The weight did not move.

Inspection of the table will show the following suggestive facts:

1. Of the eight solutions employed, sodium chloride is much the most favorable for preserving the cilia in such condition that they can do mechanical work. All the specimens in this solution were

able to do work at the end of four hours, and almost one-half of them could still move the weight perceptibly at the end of twenty-four hours.

2. Arranged in the order of most favorable action upon the working power of the cilia, these solutions stand sodium, strontium, potassium, calcium, ammonium, magnesium, lithium, barium.

3. Most of the solutions exert a stimulating action when first applied, as can be seen by comparing the first and second columns of figures. The most stimulating is ammonium; it is also one of the most injurious.

4. Magnesium and lithium do not stimulate. Lithium, especially, has from the first a markedly depressing effect.

Duration of Ciliary Activity in the Various Solutions.

This method of comparison possesses several advantages over the preceding one. Chief among these is the fact that several specimens can be made from the throat of one animal, and these placed in different solutions can be used as checks upon one another; while in the study of work done each animal furnishes material for a single experiment only. Again, the observations are made by means of the microscope directly upon the cilia themselves, and there is no question as to the possibility of error through change in the quantity or quality of mucus, or through drying during observation. Then, too, as the evidence presented will show, it is possible for a solution, by acting upon the cement-substance, to loosen the cells from their attachment, and thus destroy their power of moving a load without at the same time exercising a markedly injurious action upon the cilia.

On the other hand, there are certain decided disadvantages. The cilia cannot be observed *in situ* with sufficient certainty, and teasing off portions for examination leaves the specimen each time in less and less favorable condition. Besides this, not all portions of the epithelium act with equal vigor,[1] and, under like favorable conditions, some portions probably live longer than others. Another disadvantage lies in the fact that, in almost every preparation, some cells are

[1] Grützner: Physiologische Studien von Grützner und Luchsinger, 1882, p. 15. (Festschrift für Valentin.)

very greatly more resistant than others, and hence it is difficult to say when ciliary action has ceased to be general.[1]

In the experiments here recorded, each specimen was cut into a number of pieces, usually either four or six, and each piece was put into a different solution. The sets of experiments were arranged so as to overlap, and by some solution common to both, usually sodium chloride, act as controls upon one another.

In order to remove adherent liquid each piece was rinsed in a quantity of the solution in which it was to be placed, the liquid was then poured off and a supply of clean solution added. Observations were made from time to time by teasing off small portions of the ephithelium, and examining with a Leitz No. 7 objective. In these examinations it was necessary to keep in mind the fact mentioned by Engelmann,[2] that cilia which have come to stand still in a salt-solution became active when stimulated by teasing, or by exposure to the air, but quickly return to the inactive state.

It is not easy to give the results of these experiments without repeating in tiresome detail the notes upon the various preparations, but the main results are sufficiently shown in Table IV.

TABLE IV.

Average duration of activity of the cilia of the frog's œsophagus in pure $\frac{1}{8}$ mol. solutions.

LiCl	25 to 35 hours.	$MgCl_2$	28 to 35 hours.
NH_4Cl . . .	20 " 33 "	$CaCl_2$	25 " 35 "
NaCl	40 " 60 "	$SrCl_2$	38 " 48 "
KCl	25 " 30 "	$BaCl_2$	30 " 40 "

Inspection of the above table shows:

1. That the cilia live much longer in a pure sodium-chloride solution than in a solution of any other single salt.

2. That the chlorides of strontium and barium stand next to sodium in power of maintaining ciliary activity.

3. That no marked difference exists in the effect of lithium chloride, magnesium chloride, and calcium chloride.

[1] A similar fact was observed by BARRAT in his study of the effect of acids and alkalies on living Paramœcium. He found that in a tube containing about sixty specimens, almost all of which were dead in fifteen minutes, there were likely to be two or three individuals which would remain alive from thirty minutes to an hour or more. Zeitschrift für allgemeine Physiologie, 1904, iv, p. 441.

[2] ENGELMANN: Jenaische Zeitschrift, 1868, iv, p. 321.

4. That no marked difference exists in the two most unfavorable salts, potassium chloride and ammonium chloride.

If these eight solutions are arranged in the order of their power to maintain ciliary activity, they would probably stand as follows: Sodium, strontium, barium, magnesium, lithium, calcium, potassium, ammonium.

Comparison of the Results of the Two Methods.

If we compare this order with the order obtained in the experiments upon the work of the cilia, it will be seen that they are markedly different.[1] Such a difference, however, is not surprising when considered in connection with certain facts in regard to rhythmical contraction of muscle. In one solution the tissue may retain its irritability for a considerable time without making any rhythmic contractions. In another rhythmic contractions may occur for a time, and the muscle lose its irritability. An apical strip of tortoise ventricle placed directly in serum or Ringer's fluid usually fails to show spontaneous rhythmic contractions, but retains its irritability for many hours.[2] On the other hand, if a strip is placed in a pure solution of sodium chloride, it goes for a time into a state of rhythmic activity; but its contractions decrease in energy until irritability is finally lost in a much shorter time than that of the strip which has remained quiescent in Ringer's. It is certainly possible that a solution which causes a tissue to go for a time into a state of intense metabolism may at the same time act unfavorably upon the continued life of the tissue. It seems to me that in the study of rhythmic contractility there has not been so far sufficient discrimination between conditions which favor the retention of irritability and conditions which by reason of the irritability bring about periodic activity. In the analysis of the conditions of rhythmic action, three factors need to be looked for, namely, (1) those which maintain the irritability of the

[1] In order to test the possibility that the preparations for work were in part shielded from rapid diffusion by pinning to the paraffin block, and hence that the two sets of results might not be comparable, several control experiments were made. Each preparation was cut into equal longitudinal strips. One of the strips was pinned down to a paraffin block and placed in a dish of solution as in the experiments on work; the other strip was immersed in another dish of the same solution. No essential difference was found in the duration of life in the two sets of preparations.

[2] Greene: This journal, 1899, ii, p. 125.

tissue ; (2) those which, aside from any nutritive function in the ordinary sense, enter into the process by which mechanical energy (contraction) is produced; and (3) those which act as the stimulus and cause the discharge of the contraction-process.

It is possible that in the application of a solution of a single salt to the ciliated cell these factors would be unequally influenced, and that different salts would act most markedly upon different factors. We should then expect to find that one salt, while maintaining the cell in a living condition for a considerable length of time, might very materially lessen the amount of work done, by not furnishing the necessary condition of stimulation. Another salt could cause vigorous action until the store of available energy was exhausted. Or, to state the matter in general terms, the difference between the actions of the various salts is qualitative as well as quantitative. And even when there is little quantitative there may be considerable qualitative difference. As regards effect upon the duration of action and the working power of the frog's ciliated epithelium, lithium and ammonium both stand very low; but it is evident that they do not both act in the same way, for lithium depresses, while ammonium stimulates. Lithium is not quite so injurious as ammonium, but we cannot conclude that this is because it does not stimulate, for sodium and strontium, which are much more favorable, also stimulate. It is fair to conclude then that lithium and ammonium owe their characteristic action to effects upon different factors in the life of the cell.

In comparing the order of salts as to favorable action upon working power and duration of life of the cilia, the most striking difference is shown in the position of barium, which is among the most favorable to continued activity, and the most unfavorable of all in its influence upon the working power. In this case the explanation is simple. All the specimens which stand for a time in a salt-solution undergo a certain amount of maceration, by which single cells or cell-groups become loosened from their connection with other cells. In some of the solutions this is insignificant in amount, but in barium chloride the effect is extreme. Within a few hours multitudes of the ciliated cells are loosened from one another and float free in the liquid, the cilia remaining vigorously active. Under the microscope one often sees these cells swimming actively about, or spinning round and round by the action of the cilia, and exhibiting a marked resemblance to free-swimming protozoa. It is evident that as soon as this process of separation of the cells begins, the working power of the epithelium

as a whole must be exceedingly reduced, while the life of the individual cells is not necessarily affected. This action of barium is particularly instructive, for it shows an instance of marked action upon one part of the tissue — the cement-substance between the cells — without any very great effect upon the cilia. The comparative position of potassium in the two orders referred to is, next to barium, the most noticeable. In this case no external cause for the difference was discovered, but it is not improbable that there is here as sharp a differentiation in the action upon the internal constituents of the cell as that which occurs more externally in the case of barium.

Interpretation of Results.

I wish now to consider whether the results obtained can be expressed in terms of any general relation between the properties of the solutions employed in the foregoing experiments.

1. **Molecular weights.** — Weinland[1] concluded from experiments with a considerable number of solutions, including strong solutions of six of the eight salts which I have employed, that, with few exceptions, the stimulating action upon the cilia of the epithelium of the frog's œsophagus has a direct relation to the molecular weight; the higher the molecular weight, the more stimulating and also the more injurious the substance. With $\frac{1}{8}$ mol. solutions, however, this is certainly not the case. In Tables III and IV the salts are arranged in the order of their molecular weights, and this is very far from the order of their physiological effect. Ammonium chloride, the most injurious salt in the series, stands next to sodium chloride, the most favorable. Barium chloride, with its very high molecular weight, is more favorable to continued life of the cilia than lithium chloride, with its low molecular weight. Strontium chloride is more favorable than magnesium chloride. In fact, so far as these eight salts are concerned, absolutely no relation between molecular weight and physiological action can be found.

2. **Valence.** — The question of the possibility of a relation between toxic action and valence of the cation has been discussed by Loeb,[2] Mathews,[3] and others.

[1] Weinland: Archiv für die gesammte Physiologie, 1894, lviii, p. 131.

[2] Loeb: Archiv für die gesammte Physiologie, 1901, lxxxviii, p. 68; This journal, 1902, vi, p. 429; Loeb and Gies: Archiv für die gesammte Physiologie, 1902, xciii, p. 246.

[3] Mathews: This journal, 1905, xii, pp. 424 and 438.

Inspection of Tables III and IV shows that in the action of these salts upon the frog's epithelium no direct relation exists between valence and the physiological action upon the ciliated cells. Strontium chloride stands next to sodium chloride. Magnesium chloride and lithium chloride have nearly equal effects.

3. **Solution-tension.** — Evidence of a relation between physiological action and solution-tension has been presented by Mathews.[1] According to this, we should expect that, as shown by Table IV, sodium, barium, and strontium would be among the more favorable, and calcium, lithium, and magnesium among the more injurious. But potassium should be the most favorable of all. Barium should be more favorable than strontium, and magnesium more injurious than lithium. If we apply the correction which Mathews[2] suggests, we do not yet bring them into the order of the experiments, and are led to the conclusion that the formula is not applicable to the ciliated epithelium of the frog's throat.

4. **Probability of the existence of a direct relation.** — If we could regard living protoplasm as a definite compound possessed of clearly marked diagnostic properties, we might well seek to find a direct relation between this compound and a given series of inorganic salts, and we might have reason to expect that the salts constituting the series would be active in proportion to certain definite properties. We should also expect to find that results obtained for the cilia of one organism would hold good for another, and that principles which were found applicable to these would hold for other forms of protoplasm, as nerve, muscle, or developing eggs. If, on the other hand, living protoplasm is a mixture rather than a definite compound, a mixture containing carbohydrates, lipoids, and proteids of more than one kind, and if the proteid-mixtures are different in different organisms and different tissues, we cannot expect to find a general formula which will express the physiological action of such a series of salts. A comparison of the effect of the chlorides of sodium, potassium, and magnesium upon cilia will throw some light on this question.

According to Lillie's observations a pure sodium-chloride solution stops ciliary action almost instantly in the larvæ of Arenicola[3] and in a short time the cilia become completely dissolved. In Polygor-

[1] Mathews: This journal, 1904, xi, p. 481.
[2] Mathews: This journal, 1904, xi, pp. 485–488.
[3] Lillie: This journal, 1901, v, p. 62.

dius[1] the same kind of action occurs, but not quite so promptly. In both of these, magnesium chloride[2] and potassium chloride act much more favorably, maintaining ciliary motion for a considerable time. In the frog's epithelium we have seen that a very different relation exists; and hence we have the right to infer that the protoplasm of the cilia in the two instances is of dissimilar composition. It may be objected to the above comparison that the two kinds of cilia mentioned are adapted to life in very different media, but this objection is an admission of the existence of different properties with a probable difference of composition, and hence of reaction to inorganic salts. Comparing those cilia, however, which are adapted to the same media, we still find suggestive differences. For Parker[3] has found that the cilia of Metridiam, which, like Arenicola, live normally in sea-water, may be retained in good condition for many hours in a pure solution of sodium chloride.

It is evident that the line of reasoning just presented may well be extended to other structures than cilia. Solutions which allow ciliary action to continue render muscular contractions impossible[4] and muscular contractions can occur in solutions which destroy ciliary activity.[5]

In each case the reaction depends upon the composition of the tissue, as well as upon the inorganic reagent. The properties of the series of protoplasmic structures must be considered, as well as those of the inorganic salts employed. If this is true, no general formula can be constructed from series on either side without taking into consideration the individual properties of the entire series on the other side.

Summary.

The results of the experiments with the chlorides of lithium, ammonium, sodium, potassium, magnesium, calcium, strontium, and barium in $\frac{1}{8}$ mol. solutions upon the ciliated epithelium of the frog's œsophagus may be briefly summarized as follows:

1. Of the eight salts examined, sodium chloride is the most favorable to the prolonged life of the cells and to the preservation of their power to do mechanical work.

[1] Lillie: This journal, 1902, vii, p. 41.
[2] Lillie: This journal, 1904, x, p. 422.
[3] Parker: This journal, 1905, xiii, p. 6.
[4] Loeb: This journal, 1900, iii, p. 336.
[5] Lillie: *Loc. cit.*

2. Arranged according to their effects upon the working power of the cilia, their order is not the same as when arranged according to effects upon duration of cell-life.

3. The physiological action of these salts upon the frog's ciliated epithelium bears no direct relation to valence of the cations or to molecular weight.

4. In general, salts of higher solution-tension are more favorable than those of lower; but the order of favorable action of the individual salts does not agree with the order of their solution-tensions.

FURTHER OBSERVATIONS ON THE CATALYTIC DECOMPOSITION OF HYDROGEN PEROXIDE.

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THE recent work of Cohnheim [1] on the glycolytic action of the expressed juices of the pancreas and muscle has aroused great interest in consequence of the light it seems to throw on the nature of diabetes and the rôle of the pancreas in carbohydrate metabolism. Cohnheim showed that the expressed juices of the pancreas and muscle have individually very little glycolytic activity, but mixtures of the two in the proper proportion possess very marked power to destroy glucose. Rahel Hirsch,[2] working independently, found that a similar relation obtains between the liver and pancreas. Arnheim and Rosenbaum [3] (using different methods) found that the pancreas, liver, and muscle possess individually distinct glycolytic activity, but that combining pancreas with the other tissues causes marked acceleration. Combining liver with muscle causes no acceleration. Various observers have found that many organs, namely, pancreas, muscle, liver, ovary, kidney, etc., possess some glycolytic activity. All this work seems to indicate that the various tissues possess some slight power to destroy glucose, and that the pancreas furnishes an internal secretion which greatly increases the glycolytic action of the tissues. The great importance and practical bearing of this work make it important to check the results in every way. The nature of the glycolytic process has not been cleared up in the least by these recent contributions, and we are at present entirely ignorant of the fate of the sugar in the glycolytic process. It occurred to the writer that it would be interesting in this connection to study the effect of mixing extracts of different organs on some other better understood chemical process which all

[1] Cohnheim: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 336; 1904, xlii, p. 401.

[2] Rahel Hirsch: Hofmeister's Beiträge, 1904, iv, p. 535.

[3] Arnheim and Rosenbaum: Zeitschrift für physiologische Chemie, 1903, xl, p. 220.

the tissues are capable of bringing about to some degree. The catalysis of hydrogen peroxide was selected for this purpose, because the determinations are rapid and fairly accurate, and this reaction was being studied in other connections. The organs used were obtained from dogs and pigs; the extracts which were at first employed being prepared as follows: 10 gm. of the freshly removed tissue was thoroughly ground up with sand, and extracted with water. This was

SERIES 1.

LIVER AND PANCREAS EXTRACT (10 PER CENT).

Time in seconds.	1. 1 c.c. liver. 4 c.c. water.	2. 4 c.c. pancreas. 1 c.c. water.	3. Sum of 1 and 2.	4. 1 c.c. liver. 4 c.c. pancreas.
15	5.4	1.0	6.4	27.7
30	8.0	1.4	9.4	38.6
45	10.1	1.7	11.8	50.2
60	11.9	2.1	14.0	60.2
75	13.5	2.4	15.9	64.9
90	15.0	2.8	17.8	68.1
105	16.3	3.2	19.5	69.6
120	17.4	3.5	20.9	70.3

then strained through cloth and diluted to 100 c.c. and toluene added. These extracts were sometimes employed for several days without much change being noted. They are always faintly acid. The method of following the reaction was the same as that used by Kastle and the writer.[1] 5 c.c. of hydrogen peroxide were placed in a small bottle, which was in turn placed in a larger one containing the extract. The larger bottle was then connected with a gas burette. The experiment was started by overturning the smaller bottle without opening the apparatus, thus bringing the hydrogen peroxide in contact with the extract. At intervals of fifteen seconds, the volume of the oxygen liberated was read, and is recorded in cubic centimetres in the

[1] LOEVENHART and KASTLE: American chemical journal, 1903, xxix, pp. 397, 563.

tables which follow. The duration of each experiment was two minutes. The gas volumes are not corrected for temperature and pressure, as only relative results were required, and all of the experiments of each series were performed within a few hours. Moreover, no attempt was made to draw conclusions from small differences, only striking results being considered. During the experiments, all of which were performed at room-temperature, the bottle was shaken continuously in order to get uniform results. The hydrogen peroxide employed was the commercial Oakland "Dioxogen," which has an acidity varying from $\frac{n}{70} - \frac{n}{90}$ in different specimens. 5 c.c. of this hydrogen peroxide solution were used in each experiment, and this amount yields from 55–74 c.c. of oxygen on complete decomposition.

The first experiments (Series 1) showed that by mixing liver and pancreas extracts the reaction is accelerated very remarkably. By comparing Columns 3 and 4 it will be seen that the reaction proceeded from 230 per cent to 330 per cent faster when the liver and pancreas acted together than when they acted separately.

From Series 2 we see that the combination muscle and liver is practically as active as pancreas and liver. Another series of experiments

SERIES 2.

LIVER AND MUSCLE.

Time in seconds.	1. 1 c.c. liver. 4 c.c. water.	2. 4 c.c. muscle. 1 c.c. water.	3. Sum of 1 and 2.	4. 1 c.c. liver. 4 c.c. muscle.
15	5.4	1.3	6.7	27.8
30	8.0	1.9	9.9	38.7
45	10.1	2.6	12.7	48.5
60	11.9	3.2	15.1	55.8
75	13.5	3.8	17.3	61.3
90	15.0	4.3	19.3	65.3
105	16.3	4.9	21.2	67.5
120	17.4	5.3	22.7	68.7

carried out with 1 c.c. liver extract, 2 c.c. pancreas and 2 c.c. muscle extracts acting simultaneously give practically the same result as that

recorded above for the liver and pancreas and for the liver and muscle. It was found that combining pancreas and muscle caused only a very slight acceleration. This result is quite contrary to what has been observed in the work on glycolysis. Arnheim and Rosenbaum found that mixtures of liver and muscle show no acceleration of the glycolytic process, whereas mixtures of pancreas and muscle showed marked acceleration. Cohnheim[1] reports in his last communication that the activating substance of the pancreas in the glycolytic process is not destroyed by boiling. Some of the pancreatic extract used in the above experiments was boiled and filtered from the coagulated masses. It was found that this boiled extract which, to be sure, had no activity in itself, was capable of accelerating the liver to the same degree as that shown above by the unboiled extract, as is seen in Series 3.

SERIES 3.

Time in seconds.	1 c.c. liver. 4 c.c. water.	1 c.c. liver. 4 c.c. boiled pancreas extract.
15	6.1	22.2
30	8.2	34.4
45	10.6	45.6
60	12.7	55.0
75	14.4	61.2
90	15.8	65.7
105	17.4	68.1
120	18.5	69.2

The reaction under consideration resembles the glycolytic process in that the accelerating effect of the pancreas is not destroyed by boiling. Cohnheim[2] found that increasing the amount of the expressed juice of pancreas with which a given amount of muscle juice was mixed, causes an increase in the rate of glycolysis up to a certain point, beyond which a further increase in the amount of pancreas juice markedly diminishes the velocity of the reaction. No such optimum for the amount of pancreas acting has been found for

[1] COHNHEIM: Zeitschrift für physiologische Chemie, 1904, xlii, p. 401.

[2] COHNHEIM: *Loc. cit.*

the reaction here studied. Within ordinary limits, an increase in the amount of pancreas acting causes an increase in the rate of the catalysis.

In order to see if any peculiar relationship exists between any of the organs with reference to this reaction, extracts of the liver, adrenal, lung, muscle, brain, spleen, kidney, and pancreas of a dog were prepared, and the action of each tested on the other. The only noteworthy result was that in every case where two extracts were mixed together, some acceleration was noted, though in several instances it was very slight. We next determined to find, if possible, the cause of the acceleration, and with this in view a clear solution of the enzyme was prepared. This was made by using a modification of Jacoby's uranyl acetate method similar to that recommended by Rosell.[1] 100 c.c. of a 10 per cent extract of the liver or pancreas was treated with 20 c.c. of a saturated solution of uranyl acetate, and the mixture neutralized by adding a few drops of a saturated solution of sodium carbonate and sodium phosphate. After standing a few moments, 5 c.c. of a saturated solution of sodium phosphate were added, and the mixture centrifugalized and then filtered. The water-clear filtrate thus obtained was always found to possess strong catalytic activity whenever the original extract was active. Boiling causes it to lose all activity. This solution can be kept several days by adding a few drops of toluene, and only requires to be filtered from the precipitate, which slowly appears. These clear extracts contained only a very small amount of proteid. They were always amphoteric or very slightly alkaline in reaction; whereas the turbid extracts were always slightly acid. The results obtained with the turbid extracts were confirmed with the clear extracts. It was found that not only can boiled pancreas accelerate the decomposition by liver, but the boiled liver itself can accelerate the action of fresh liver. These points are brought out in Series 4. In all experiments which follow, the clear liver extract was diluted with $1\frac{1}{2}$ volumes of distilled water in order to reduce its activity sufficiently to show the acceleration well. In the experiments in which the boiled liver extract was used, the undiluted 10 per cent extract was employed in order to have the conditions exactly comparable to the experiments with the pancreas.

By comparing Columns 3 and 4, it will be seen that the reaction is

[1] Rosell: Inaugural dissertation, Strassburg, 1901 (cited by Magnus: Zeitschrift für physiologische Chemie, 1904, xlii, p. 149).

accelerated from 100 to 300 per cent by allowing the liver and pancreas to act simultaneously. Columns 5 and 6 show that boiled liver and boiled pancreas have about the same power to accelerate the catalysis by fresh liver that fresh pancreas has. In these experiments the action of the liver was increased about seven times in the earlier readings. By comparing Columns 2, 7, and 8, it will be observed that neither the boiled liver nor the boiled pancreas is capable of accelerating the catalysis by fresh pancreas to any

SERIES 4.

Time in seconds.	1. 1 c.c. liver. 1 c.c. water.	2. 1 c.c. pancreas. 1 c.c. water.	3. Sum of 1 and 2.	4. 1 c.c. liver. 1 c.c. pancreas.	5. 1 c.c. liver. 1 c.c. boiled pancreas.	6. 1 c.c. liver. 1 c.c. boiled liver.	7. 1 c.c. pancreas. 1 c.c. boiled liver.	8. 1 c.c. pancreas. 1 c.c. boiled pancreas.
15	2.6	3.1	5.7	21.8	18.8	20.9	4.4	5.0
30	4.8	4.3	9.1	31.0	26.0	28.8	5.5	5.7
45	7.2	5.0	12.2	38.0	31.6	33.4	6.3	6.2
60	9.3	5.8	15.1	43.2	37.4	38.8	6.8	6.9
75	11.4	6.4	17.8	48.1	41.8	42.9	7.5	7.3
90	13.1	7.1	20.2	51.6	45.7	46.2	8.0	7.9
105	15.2	7.7	21.9	53.4	48.6	48.8	8.6	8.4
120	17.4	8.3	25.7	55.5	51.1	50.9	9.1	9.0

extent. Thus we see that the action of boiled liver and boiled pancreas is the same, namely, they both cause a very great acceleration in the action of liver, but neither is capable of acclerating the action of pancreas to any extent. This indicated that liver and pancreatic catalysis may be different, and some experiments were made in order to further test this point. With this in view the action of several substances on catalysis by liver and pancreas extracts was investigated in the hope of finding that certain substances would accelerate the one and retard the other, and thus confirm the non-identity of the two catalases. Solutions of sodium sulphate, sodium thiosulphate, thiourea, ammonium sulphocyanate, and ammonium nitrate were employed, the results being given in Series 5 and 6. In each experiment 1 c.c. of the extract and 1 c.c. of the solution

to be tested were used. Experiments (1 and 7) with 1 c.c. water is given for comparison.

A comparison of the corresponding columns shows that qualitatively the effect of these substances on the catalysis by liver and pancreas extracts is the same, but certain quantitative differences are very

SERIES 5.

LIVER EXTRACT (4 PER CENT)

Time in seconds.	1. Water.	2. n $Na_2S_2O_8$.	3. $\frac{2}{5}$ n Na_2SO_4.	4. n NH_4NO_3.	5. n NH_4CNS.	6. $\frac{n}{5}$ thiourea.
15	1.0	9.7	0.8	0.1	0.1	1.4
30	1.6	11.9	0.8	0.2	0.1	3.4
45	2.0	14.0	2.0	0.3	0.2	5.3
60	2.6	16.0	2.4	0.3	0.2	6.5
75	3.0	18.1	2.9	0.3	0.2	7.6
90	3.5	20.1	3.3	0.3	0.3	8.5
105	4.0	22.4	3.7	0.4	0.3	9.3
120	4.4	24.2	4.1	0.4	0.3	9.9

SERIES 6.

PANCREAS EXTRACT (10 PER CENT).

Time in seconds.	7. Water.	8. n $Na_2S_2O_8$.	9. $\frac{2}{5}$ n Na_2SO_4.	10. n NH_4NO_3.	11. n NH_4CNS.	12. $\frac{n}{5}$ thiourea.
15	1.6	5.7	1.7	0.2	0.2	2.2
30	2.7	6.9	2.7	0.2	0.3	3.7
45	3.7	8.1	3.5	0.3	0.4	4.9
60	4.5	9.3	4.3	0.4	0.4	5.8
75	5.4	10.6	5.1	0.4	0.4	7.3
90	6.2	11.8	5.8	0.4	0.4	7.3
105	7.0	13.2	6.6	0.5	0.5	7.9
120	7.7	14.5	7.3	0.6	0.5	8.4

marked. Thus the action of the liver extract is accelerated from 400 per cent to 800 per cent by sodium thiosulphate, whereas pancreas extract is only accelerated from 100 per cent to 250 per cent. The thiourea also accelerates the action of the liver more than it does the pancreas extract. No conclusion as to the identity or non-identity of hepatic and pancreatic catalase could be drawn from these experiments. The effect of temperature on the activity of the two extracts was studied. It was found that the activity of liver extract at 1° is 41.5 per cent of its activity at 20°, and the activity of the pancreas at 1° is 48.5 per cent of its activity at 20°. Hence temperature affects the activity of liver and pancreas to about the same extent, and the writer concluded that the enzymes are probably identical, and that the differences observed are due to differences in the environment in the liver and pancreas.

SERIES 7.

Time in seconds.	1. 0.5 c.c. liver. 4.5 c.c. water.	2. 1.0 c.c. liver. 4.0 c.c. water.	3. 2.0 c.c. liver. 3.0 c.c. water.	4. 3.0 c.c. liver. 2.0 c.c. water.	5. 4.0 c.c. liver. 1.0 c.c. water.	6. 5.0 c.c. liver. No water.
15	0.5	1.0	5.3	22.4	38.6	50 6
30	0.8	1.3	7.9	32.4	52.6	65.8
45	0.9	1.6	10.2	39.8	61.0	70.8
60	1.0	1.8	12.3	45.9	65.4	71.7
75	1.0	2.0	14.4	51.0	67.6	71.7
90	1.1	2.2	16.3	55.5	68.4	71.6
105	1.2	2 4	17.9	59.3	68 5	..
120	1.3	2 6	18.6	62.2	68.6	..

The fact that boiled liver can accelerate the catalysis by liver, as has been already pointed out, proved that the liver must contain besides the catalase a substance which is not destroyed by heat, and which is capable of accelerating the action of the liver catalase. As the liver contains a substance which can accelerate its own catalase, it was thought possible that by increasing the amount of liver acting, more than a corresponding increase in activity might be noted. Ac-

cordingly experiments were made in which the concentration of the liver extract was varied, while the concentration of the hydrogen peroxide remained constant. The results are seen in Series 7 (4 per cent liver extract used).

Columns 1 and 2 show that for small quantities of the extract the rate of the oxygen liberation is proportional to the amount of extract acting. When larger amounts of extract are used, however, an increase in the amount of extract acting causes far more than a proportional increase in the rate of oxygen evolution. Thus by comparing the first readings in Columns 1 and 6, it will be seen that the reading in Column 6 is ten times as large as it would be if the liver acted in proportion to the amount present. A similar series was carried out with a 10 per cent pancreatic extract, the results of which follow :

SERIES 8.

Time in seconds.	1. 1 c.c. pancreas extract. 4 c.c. water.	2. 2 c.c. pancreas extract. 3 c.c. water.	3. 3 c.c. pancreas extract. 2 c.c. water.	4. 4 c.c. pancreas extract. 1 c.c. water.	5. 5 c.c. pancreas extract. No water.
15	0.5	1.3	4.1	5.9	8.3
30	0.7	1.9	5.7	8.0	11.5
45	0.8	2.5	7.2	9.9	14.2
60	0.9	3.1	8.5	11.8	17.8
75	1.0	3.7	9.8	13.6	19.8
90	1.1	4.3	11.0	15.3	22.5
105	1.2	4.9	12.2	17.1	25.2
120	1.3	5.5	13.5	18.6	28.0

Thus an increase in the amount of pancreas acting alone also causes more than a corresponding increase in the rate of the catalysis, but the figures are not so striking as in the case of the liver. This was expected from the fact that the action of pancreas is not stimulated by boiled pancreas to anything like the extent that the action of liver is accelerated by boiled liver.

G. Senter[1] has shown that the action of the catalase of the blood on hydrogen peroxide at 0° follows the equation of a reaction of

[1] Senter : Zeitschrift für physikalische Chemie, 1903, xliv, p. 257.

the first order, but at ordinary temperature the value 0.4343 K constantly diminishes as the reaction proceeds. This he attributed very properly to the oxidation or at least destruction of the enzyme. Issajew[1] has recently found that the action of yeast catalase is also a reaction of the first order. The results of the experiments reported above could not be expected to yield a constant when applied to the equation of a reaction of the first order, because they were performed at room-temperature, and the reaction proceeded too rapidly under the conditions of the experiment. The value 0.4343 K in the above experiments often diminishes 50 per cent as the reaction proceeds. As the reaction has been previously shown to be one of the first order, however, the value 0.4343 K was calculated from the above experiments and by averaging the mean velocity for a given experiment determined. The average values of 0.4343 K in the series, with increasing amounts of liver (p. 178), are as follows:

c.c. liver acting.	0.4343 K.	c.c. liver acting.	0.4343 K.
0.5	0.000109	3.0	0.0081
1.0	0.00021	4.0	0.017
2.0	0.0015	5.0	0.037

If the coefficient of velocity were proportional to the amount of liver acting, the value for 5 c.c. liver extract should be ten times as great as for 0.5 c.c., whereas we find it to be 339.5 times as great. Hence the reaction proceeds 33.95 times as fast when 5 c.c. of liver extract are employed as it would have done if the coefficient of velocity were proportional to the amount of liver acting. The results here obtained offer an explanation of the results of Issajew. Issajew found that increasing the amount of enzyme acting did not cause a proportional increase in the coefficient of velocity, but after dialysing the enzyme solution, an increase in the amount of enzyme acting causes more than a proportional increase in the value of K. Issajew considered, among other explanations, the possibility that during dialysis substances were removed which affected in some way the reaction. This is undoubtedly the correct explanation. Pawlow and Parastschuk[2] have recently shown that by varying the conditions, the velocity of the action of rennin may be proportional to the amount of the enzyme acting, or to its square, or its square root. It is, therefore, important,

[1] Issajew: Zeitschrift für physiologische Chemie, 1904, xlii, p. 102.

[2] Pawlow and Parastschuk: Zeitschrift für physiologische Chemie, 1904, xlii, p. 415.

in studying the velocity of enzyme action, to remember that we never have present merely the enzyme and the substance acted upon, but also other substances which often greatly influence the velocity of the reaction.[1]

In the hope of finding the cause of these remarkable accelerations, it was decided to vary the conditions of the experiments. This was done in the first place by neutralizing the slight acidity of the hydrogen peroxide. 5 c.c. of the hydrogen peroxide required for neutralization from 1 c.c. to 1.5 c.c. of $\frac{n}{20}$ NaOH, and, in the experiments which follow, 5 c.c. of the hydrogen peroxide were placed in the small bottle, previously described, together with the amount of $\frac{n}{20}$ NaOH required to neutralize it. The experiments previously described with the acid hydrogen peroxide were now repeated with the neutralized solution under the same conditions.

The results were as follows:

SERIES 9. — Effect of increasing the amount of liver acting on the neutralized hydrogen peroxide.

Time in seconds.	1. 1 c.c. liver. 4 c.c. water.	2. 2 c.c. liver. 3 c.c. water.	3. 3 c.c. liver 2 c.c. water.	4. 4 c.c. liver. 1 c.c. water.	5. 5 c.c. liver. No water.
15	7.5	15.3	21.1	27.0	33.1
30	10.0	20.5	30.7	37.8	43.7
45	12.0	24.7	37.1	43.9	48.2
60	13.8	28.7	42.4	47.4	49.4
75	15.5	32.9	45.9	48.9	49.5
90	17.3	36.4	48.2	49.2	49.5
105	19.1	39.9	49.2	49.2	..
120	20.7	42.7	49.5	49.2	..

For purposes of comparison, only the early readings can be used in a reaction proceeding as rapidly as this one, and by examining the first readings in the above tables it will be seen that the rate of oxygen evolution is proportional to the amount of liver acting, whereas with the acid peroxide the velocity of the reaction increased far more rapidly than the increase in the amount of extract acting would indicate.

[1] Since this article was written, BACH has expressed the same opinion (Berichte der deutschen chemischen Gesellschaft, 1904, xxxvii, p. 3785).

This table shows that with the neutralized hydrogen peroxide an increase in the amount of pancreatic extract acting does not cause

SERIES 10. — Effect of increasing the amount of pancreatic extract acting on the neutralized hydrogen peroxide.

Time in seconds.	1. 1 c.c. pancreas extract. 4 c.c. water.	2. 2 c.c. pancreas extract. 3 c.c. water.	3. 3 c.c. pancreas extract. 2 c.c. water.
15	2.0	3.6	5.2
30	2.6	4.8	6.8
45	3.2	5.9	8.2
60	3.7	6.9	9.5
75	4.2	7.9	11.2
90	4.8	9.0	12.8
105	5.3	10.1	14.4
120	5.8	11.1	15.9

quite a corresponding increase in the rate of oxygen evolution. It was next determined whether the pancreas could accelerate the catalysis by liver when the peroxide had been previously neutralized. The results are seen in Series 11.

A comparison of Columns 3 and 4, and of 7 and 8, shows that whereas the combined action of pancreas and liver acting on acid peroxide causes an acceleration of about 200 per cent, practically no acceleration is noted if the peroxide is neutral. It is perfectly evident from all the facts that the power of the pancreatic extract to accelerate the catalysis of acid hydrogen peroxide by liver extract is merely due to the power of the pancreatic extract to neutralize the retarding effect of the acid contained in the commercial peroxide. It is to be noted in this connection that the pancreatic extracts employed were often slightly acid toward litmus, especially the turbid extracts originally employed, and hence it is the more interesting that they can prevent the retarding action of the acid contained in the peroxide.

It will be noted on comparing Columns 1 and 5, and also 2 and 6 of the last tables, that while the action of the liver extract was accelerated 475 per cent by merely neutralizing the hydrogen peroxide, that of the pancreas was only accelerated from 100 per cent to 200 per cent. The fact can be expressed in another way, namely, the action

of liver extract is inhibited relatively much more by acids than the action of pancreas extract is. Since both boiled and fresh liver and

SERIES 11.

ACID HYDROGEN PEROXIDE.

Time in seconds.	1. 1 c.c. liver. 1 c.c. water.	2. 1 c.c. pancreas. 1 c.c. water.	3. Sum of 1 and 2.	4 1 c.c. liver extract. 1 c.c. pancreas.
15	0.7	0.2	0.9	2.4
30	0.9	0.4	1.3	3.2
45	1.0	0.4	1.4	3.7
60	1.1	0.5	1.6	4.3
75	1.2	0.5	1.7	4.8
90	1.2	0.6	1.8	5.3
105	1.3	0.6	1.9	5.7
120	1.3	0.6	1.9	6.2

SERIES 12.

NEUTRALIZED HYDROGEN PEROXIDE.

Time in seconds.	5. 1 c.c. liver. 1 c.c. water.	6. 1 c.c. pancreas. 1 c.c. water.	7. Sum of 5 and 6.	8. 1 c.c. liver extract. 1 c.c. pancreas.
15	4.1	0.6	4.7	5.6
30	4.8	0.8	5.6	6.2
45	5.3	0.8	6.1	6.7
60	5.7	0.9	6.6	7.2
75	6.2	1.0	7.2	7.7
90	6.6	1.0	7.6	8.2
105	7.0	1.0	8.0	8.7
120	7.5	1.1	8.6	9.2

pancreas extracts are able to neutralize the retarding effect of the acid in the commercial peroxide, it is perfectly clear why the action of the

liver extract on acid hydrogen peroxide is accelerated more by the simultaneous presence of these extracts than is the pancreas extract. In those experiments in which an increasing amount of liver was used, each increase in the quantity of extract not only increased the amount of enzyme acting, but also improved the conditions for the reaction by neutralizing the effect of the acid present, and thereby causing the great acceleration noted.

As already stated, this work was undertaken to check the work on the glycolytic action of mixed tissue extracts. The absence of any specificity and the absence of Cohnheim's "Ablenkung" phenomenon prove that the reaction under consideration is not at all comparable to the glycolytic process. The results which have been reported in the above indicate, however, that great caution must be observed in drawing conclusions from experiments with mixtures of the extracts of different organs. In several cases of enzymic action, the velocity has been found to be greatly increased by mixing extracts of certain tissues with the solution containing the enzyme. It is well known that certain conditions favor the action of certain enzymes, and to conclude from such results that the inactive solution contains a peculiar "kinase" seems unjustifiable.

After treating the enzyme with the kinase, no attempt has been made, so far as I am aware, to remove the kinase, and hence we cannot be sure whether it acts on the enzyme or not. If we could not remove the hydrochloric acid from a pepsin hydrochloric acid mixture, it might be called a kinase.

It should be remembered that many of these processes are as sensitive to a change of reaction as the indicators which are used to test the reaction, and which are notoriously uncertain in these solutions. In the case of the coagulation of the blood, for instance, the accelerating effect of mixing muscle extract with serum has recently given rise to a complicated theory. The whole "kinase" conception, in the opinion of the writer, rests on a very slender and uncertain basis of fact. How simple it would be, in the reaction reported in this paper, to conclude from a few experiments that the pancreas contains a "catalokinase" or "coferment." There is, of course, no denying certain facts which have been brought out as a result of the work on the "kinases," but the view that the increased activity is due to a specific enzyme which renders kinetic the dormant enzyme, as the name kinase indicates, is an unwarranted and speculative conception resting on but few facts.

SUMMARY.

When unneutralized commercial hydrogen peroxide is used, it is found that:

(1) Pancreas extract markedly accelerates the decomposition of the peroxide by liver extract. Muscle extract has the same accelerating action.

(2) The accelerating property of the pancreas is not destroyed by boiling.

(3) Boiled and fresh liver extract greatly accelerate the decomposition of hydrogen peroxide by fresh liver extract.

(4) When neutralized hydrogen peroxide is employed, none of these accelerations are noted, and the accelerating action of these extracts is due to the fact that they neutralize the retarding action of the acid contained in the commercial peroxide. Acids inhibit the action of the pancreas extract relatively much less than they do that of liver extract, and hence the action of pancreas extract on the acid hydrogen peroxide is not so greatly accelerated by boiled or fresh liver or pancreas extract. On the other hand, sodium thiosulphate and thiourea accelerate the action of liver extract more than they do that of the pancreas extract. Temperature, however, affects the activity of liver and pancreas alike, and the differences observed are probably due to differences in the environment of the catalase in the liver and pancreas, the enzymes being probably identical.

TWITCHINGS OF SKELETAL MUSCLES PRODUCED BY SALT-SOLUTIONS WITH SPECIAL REFERENCE TO TWITCHINGS OF MAMMALIAN MUSCLES.[1]

By WALTER E. GARREY.

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PROFESSOR J. LOEB and the author tested upon frog's skeletal muscles, the action of a series of sodium salts, which precipitate calcium. This work was done as the natural sequence of Loeb's previous work[2] which demonstrated that the rhythmic twitchings which these muscles exhibit in isotonic sodium-chloride $\frac{m}{8}$ may be inhibited by very small amounts of calcium chloride. Loeb at that time advanced the view that calcium salts in the blood and tissues normally inhibit muscular twitchings, and that these salts diffuse out when the muscle *is placed in sodium chloride*. The normal "balance" of the inorganic constituents (ions) is thus disturbed. If this process could be accelerated, *e.g.*, by precipitating or inactivating the calcium salts (or other inhibiting factors), the initiation of twitches should be accelerated and the twitches become more powerful than when simply immersed in sodium chloride.

We found,[3] as was anticipated, that frog's skeletal muscles immersed in sodium oxalate, phosphate, carbonate, sulphate, fluoride, etc., exhibited powerful contractions which began at once and were decidedly rhythmic in character.[4]

Loeb's more recent work shows that a number of other salts — notably barium chloride — have an effect similar to calcium precipitants in the production of muscular twitchings. The citrate, acetate, and succinate of sodium also have an effect similar to the salts which we employed in the earlier investigations. The author has continued

[1] Details of this investigation, the results of which are summarized in this article, will be published later.

[2] LOEB, J.: Festschrift für Fick, 1899, p. 101.

[3] A complete report of this work, done in 1899, has not yet been published; *cf.* LOEB, J.: Decennial publications, University of Chicago, 1902.

[4] See BIEDERMANN: Electrophysiology (WELBY), 1896, p. 104.

these investigations independently, and a summary of the recent results follows.

Muscles of cold-blooded animals. — The twitches of frog's muscles which have been started in isotonic sodium chloride are markedly increased in force by the addition of small amounts of all the salts mentioned above as producing twitches, and twitches started in the above salts continue longer, with increased force, when the muscles are subsequently transferred to pure sodium-chloride solutions, — as most of the salts employed (*e.g.*, sodium oxalate) are violent poisons in the isotonic concentration.

The injection of calcium precipitants and "inactivators" in the form of sodium salts, into the lymph spaces, peritoneal cavity, or directly into the circulation of the frog, sets up fibrillary twitchings of the whole musculature. (These experiments confirm the work of Friedenthal.[1]) These twitchings may be inhibited by the simultaneous or subsequent injection of calcium salts, and to a lesser degree, by magnesium salts. Magnesium sulphate, although a calcium precipitant, has this inhibiting effect. The action of magnesium is therefore not unlike that of calcium.

The twitchings of frog's muscles are produced by these injections after destruction of the central nervous system, after section of the motor nerves, and after the effective injections of curare. That the twitches are purely myogenic is also shown by the fact that they may be produced after section of the nerves with degeneration of the nerve-fibres and motor end-plates. Mathews[2] has furthermore shown that the immersion of nerve-fibres in many of the above solutions does not set up contractions of the muscle until after a considerable time; muscles, however, begin twitching at once or after a very short latent period.[3]

The author extended these results obtained on the frog by testing many other muscles without finding an exception to the rule that a skeletal (striated) muscle may be made to twitch and usually with a decided rhythm in the salts mentioned above. Among the muscles

[1] Friedenthal: Archiv für Physiologie, 1901, p. 145.

[2] Mathews, A. P.: This journal, 1904, xi, p. 455. Mathews also employed curare as above. See also Biedermann: *Loc. cit.*

[3] Neurogenic twitchings of frog's muscles may be produced by exposing the spinal cord and immersing it in certain isotonic salt-solutions, *e.g.*, sodium phosphate and sulphate. These experiments are still in progress, and will be published at a later date.

tested were those from Nereis, Limulus, lobster, crayfish, earthworm, cricket, salamander, lizard, and turtle.

Mammalian muscles. — Success also attended the efforts to produce twitchings of mammalian muscles by subjecting them to isotonic solutions of the same salts as were used in the production of twitches of frog's muscles; special conditions were, however, necessary. The results are the same irrespective of the mammal from which the muscles were taken; positive results were obtained with muscles of cat, dog, guinea-pig, rabbit, and with human muscle taken in two cases from amputated limbs and in one case from an excised tumor to which the muscle was adherent. The muscles of pigeons were also found to twitch in certain salt-solutions under the conditions noted below for mammalian muscle.

In experiments with the mammalian muscle the tissue was excised from the narcotized animal, and either immersed in the solutions, or the solutions were perfused under varying pressures through blood-vessels of the muscles. The results of the two methods were identical. It was found advantageous in the immersion method to employ thin muscles, *e.g.*, platysma myoides and abdominal muscles in which all fibres are more quickly and uniformly acted on by the solutions.

None of the solutions tested produced twitches in a mammalian muscle when the temperature was lower than about 30° C. At this temperature twitches may or may not be produced, the results being variable with different animals and with different muscles from the same animal.

The optimum temperature for the production of the twitches is about the body-temperature, say between 35° and 40° C. At this temperature simple immersion suffices to induce twitches in isotonic solutions[1] of the following sodium salts: phosphate, disodium phosphate, carbonate, bicarbonate, sulphate, oxalate, citrate, succinate, acetate, and tartrate, also in lithium carbonate (saturated) and in lithium sulphate.

Many of these solutions are alkaline owing to hydrolysis, and the possibility existed of the hydroxyl ions being the causal factor in the production of the twitches of mammalian muscle. This is not the case,[2] however, for the twitches occur in neutral sodium sulphate

[1] The solutions used depressed the freezing point to −0.6° C. Equimolecular solutions ($\frac{m}{8}$) proved equally effective.

[2] MATHEWS, A. P.: This journal, 1904, xi, p. 478, shows that the stimulation of nerves by a similar series of salts is not due to OH-ions.

and even in sodium sulphate after the addition of a trace of sulphuric acid; furthermore the twitches do *not* occur in sodium chloride to which sodium hydrate has been added, — no matter what the concentration of the alkali may be. It is true, however, that a small degree of alkalinity facilitates the genesis of the twitches in these solutions while acid has the opposite effect.

In pure barium-chloride solutions of all strengths, the muscles promptly die without twitching. Neither did the muscles twitch in magnesium chloride or in magnesium sulphate.

In isotonic sodium chloride twitches could not be induced under ordinary conditions, either by changes in temperature or by the addition of alkali, as noted above.

The negative results with isotonic sodium chloride were not anticipated, for my other results had been quite in harmony with those previously obtained with frog's muscles. I decided therefore to follow out suggestions to be found in the literature relating to contractions of isolated strips of mammalian ventricular muscle, and to subject the skeletal muscles in sodium chloride solutions to an atmosphere of pure oxygen; but the experiments did not succeed; simple saturation of isotonic sodium chloride, and an atmosphere of pure oxygen, did not cause a mammalian skeletal muscle to twitch spontaneously. Neither did I succeed after oxygenation of the sodium chloride with dioxide of hydrogen, as in Lingle's experiments,[1] with strips of tortoise heart; nor did these dioxide of hydrogen-experiments succeed after neutralizing the solutions or making them alkaline.

Porter, in his heart experiments,[2] used pure oxygen under pressure, and following this lead it was easily demonstrated that intermittent twitches are instituted in isotonic sodium chloride when subjected to *oxygen at a pressure of two to three atmospheres*, when the temperature is approximately 40° C. These twitches continue from one half to three fourths of an hour; they cease, however, long before the muscle loses its irritability to induction-shocks. These experiments do not succeed at 20° C. The twitches in sodium chloride under oxygen pressure begin sooner, are stronger, and last longer, if a very small amount of sodium hydrate is present.

Similarly the twitches are more pronounced if from 1–5 c.c. of isotonic barium chloride be added to each 100 c.c. of sodium chloride:

[1] Lingle: This journal, 1902, viii, p. 79.
[2] Porter: This journal, 1898, i, p. 516.

the poisonous action of barium chloride becomes evident in the rapid death of the tissues when larger amounts are added.

The addition of small amounts of any of those salts which, under ordinary conditions induce muscular twitchings, starts the twitching sooner, or increases the force of those already started in sodium chloride under oxygen pressure.

Under these conditions the addition of very small amounts of calcium chloride or nitrate inhibits the twitching at once, and the same effect is produced, although in a lesser degree, by magnesium chloride and sulphate.

Oxygen pressure will not elicit contractions of a mammalian muscle immersed in its own blood-serum or defibrinated blood. Neither would the muscles twitch in Ringer's solution, nor in diluted sea-water isotonic with the blood.

No twitches in isotonic non-electrolytes were ever noticed. Dextrose, levulose, cane sugar, lactose, urea, and glycerin were tried.

An increase in the osmotic pressure of these non-electrolytes did not induce the twitchings; [1] the muscles simply contracted tonically, and quickly lost their irritability. Increasing the osmotic pressure of pure sodium chloride to 2 *m* occasionally called forth a few initiatory, intermittent twitches which lacked any semblance of rhythm, and the result was not at all certain. This increase in the osmotic pressure of the sodium chloride caused a rapid death of the mammalian muscle. More forceful contractions follow an increase in the osmotic pressure of pure sodium chloride when there is a high oxygen pressure, but the contractions continue for a short time only. The mammalian muscle lives longer in isotonic sea-water than in any other inorganic solution tested, *e. g.*, after an immersion of one hour the muscles will often twitch in calcium precipitants or in sodium chloride under oxygen pressure, — it is therefore an excellent solution with which to test the effects of increased osmotic pressure; it was found, however, that no change in the osmotic pressure of sea-water will suffice to induce the twitchings of mammalian muscles immersed in it. In pure sea-water ($\Delta = -1.9^{\circ}$ C.) mammalian muscles die within thirty minutes.

At best the contractions of mammalian skeletal muscles, produced by the means employed in this investigation, are feeble and lack much

[1] An occasional exception to this result was noted, *e. g.*, in one instance, a strip of muscle beat for ten minutes in 2.5 mol. solution of cane sugar.

of the decided power, regularity, and rhythmicity shown by the contractions of frog's muscles under similar conditions. This fact, and the extreme susceptibility of the mammalian tissue to the poisonous action of the salts employed, made it impossible to determine with any certainty the comparative stimulating power of the various salts.

THE RÔLE OF CERTAIN IONS IN RHYTHMIC HEART ACTIVITY.

By STANLEY R. BENEDICT.

[*Contribution from the Biological Laboratory of the University of Cincinnati.*]

THE question of the rhythmicality of heart-muscle is one which has justly received much attention. Ever since the demonstration of a relationship between heart-beat and the inorganic salts of the blood, this problem has been an especially prominent one.

In view of the fact that this paper is preliminary in nature, it does not seem desirable to give a résumé here of what has already been done upon this subject. For an account of such work I refer the reader to the papers of Green[1] and Howell,[2] where will be found a résumé of results obtained, as well as a very complete bibliography of this subject. Results heretofore obtained having a bearing upon our present discussion will be considered individually.

The most recent aspect of the problem is one developed by Howell[2] and Lingle.[3] Howell has vigorously supported the theory that calcium ions are of prime importance in heart-beat; while Lingle, influenced, no doubt, by Loeb's results with striped muscle, has as strongly championed the greater importance of the sodium ion. Martin,[4] after two years' work upon the subject, comes to the conclusion that both calcium and sodium ions are necessary, if rhythmic activity is to be developed or maintained, — a position already taken by Howell.[5]

The present work was originally commenced with the idea of supporting Howell's calcium theory, since some of Lingle's[6] conclusions from his experiments seemed to be unwarranted.

[1] GREEN: This journal, 1898, ii, p. 82.
[2] HOWELL: This journal, 1898, ii, p. 47; *Ibid.*, 1901, vi. p. 181.
[3] LINGLE: This journal, 1900, iv, p. 265; *Ibid.*, 1902, viii, p. 75.
[4] MARTIN: This journal, 1904, xi, No. 2.
[5] HOWELL: This journal, 1901, vi.
[6] LINGLE: *Loc. cit.*

The strips used were prepared from the turtle's ventricle, as described by Green.[1] Three or four strips were usually prepared from one heart, in order to obtain controls for each experiment.

The salts used were of the highest purity obtainable, being Kahlbaum's best manufacture.

The line of investigation determined upon was to use different salts (*i. e.*, other than the chlorides) of the metals found in the blood. Before many experiments had been performed, the writer was forced to a theory differing widely from either Howell's or Lingle's, in order to explain the facts observed.

The purpose of the present paper is the proposal and support of this theory, which may be formulated as follows: the direct production of rhythmic activity by means of a salt's action upon heart-muscle is due to the anion of that salt, while the chief function of the cation is apparently to maintain such a tone of the heart-muscle that it will respond to the stimulus furnished by the anion.

This theory had partially suggested itself to the writer prior to the commencement of experimental work, upon reading the following statement of Lingle's:[2] "If sodium chloride is so closely associated with the real stimulus, the sodium and chlorine ions of which it is composed must now be considered. Loeb, working on striped muscle and Gonionemus tissue, found that the sodium ion was the active agent. In heart-tissue the same seems to be true; for solutions containing sodium, but no chlorine, are able to do the work of sodium chloride, and indeed can do its work even better. Among such solutions is sodium bromide. A sodium-bromide solution equimolecular with a 0.7 per cent sodium-chloride solution, if properly used, will start rhythmic beats in heart-strips, the beats so started are generally stronger than those in sodium chloride, and moreover the rhythm lasts longer."

To the writer this statement seemed to furnish evidence in an exactly opposite direction from that for which Lingle offered it. Had the action obtained with the two salts been practically identical, Lingle's statement might indicate the importance of the sodium ion. If, however, another salt of sodium, namely, the bromide, can do the work of sodium chloride, and do this work distinctly *better* than can the chloride, we are certainly forced to believe that the rôle of the anion is not entirely a passive one, as Lingle[3] would assume, but

[1] GREEN: *Loc. cit.*

[2] LINGLE: This journal, 1900, iv, p. 272.

[3] LINGLE: *Loc. cit*

that either the chlorine ion is hurtful, and the bromine ion less so (which we need not consider), or we must assume that the chlorine ion has an active rôle which the bromine ion can perform even better.

As above mentioned, the results obtained with different salt solutions (the first used was sodium-carbonate solution, with an idea of precipitating the calcium ions) soon forced the importance of the anion into prominence. Upon applying this theory to results heretofore obtained by others, it seemed not only warranted but demanded by many of them. These will be considered below.

The experimental work done to test the validity of the statement that it is the anion which furnishes the stimulus for heart-beat was as follows.

First, the correctness of Lingle's statement that sodium bromide produces a better rhythm than does sodium chloride was ascertained by making comparative experiments with equimolecular solutions of these two salts. The results most amply bore out his assertion. Strips from the same heart immersed in sodium-bromide solution, equinormal with this, almost invariably showed a latent period shorter by ten or fifteen minutes in the latter solution, while the series of beats obtained in this solution usually lasted longer than that obtained in the sodium chloride, and the rhythm was better sustained. Clearly the anions chlorine and bromine are not entirely inactive, but, on the contrary, play some positive rôle in the phenomena of heart-beats.

An interesting result obtained in some instances (when the sodium-chloride series was unusually short) was a short renewal of the series of beats in the sodium-bromide solution after exhaustion in sodium chloride. This result also points strongly to the importance of the anion.

The action of equimolecular solution of calcium chloride and calcium bromide was next compared. The calcium-chloride solution was 0.026 per cent. If bromine ions are more stimulating than chlorine ions, we might expect to find a difference here. The results obtained showed a decided difference. When immersed in pure calcium-chloride solution (0.026 per cent), ventricular strips usually pass almost immediately into a greatly increased tone, from which recovery will usually not take place upon subsequent immersion in sodium-chloride solution. This change of tone in calcium-chloride solution is usually (not always) accompanied by a short series of irregular, powerful contractions.

In calcium-bromide solution, equimolecular with 0.026 per cent solution of calcium chloride, the strip also shows the change of tone; but this change is practically always accompanied by a series of beats lasting from ten minutes to half an hour, — when, as we should expect, the muscle passes into the so-called calcium rigor. The beats obtained in the bromide solution begin after a shorter latent period, are more regular, and last longer than those obtained in the calcium-chloride solution. In a few instances a series of beats somewhat resembling a sodium-chloride series has been obtained in the calcium-bromide solution, *i. e.*, the beats began at a minimum, and gradually increased in intensity, the series occupying over forty-five minutes. The controls in calcium chloride did not show this long, graduated series, nor have I ever been able to obtain it in this latter solution.

The difference in the action of the chlorides and bromides of sodium and calcium can be accounted for only by bestowing upon the anion an active rôle hitherto not ascribed to it. That the function of direct stimulation to rhythmic beats belongs to the anion and not to the cation, will, it is believed, be conclusively shown by considering the behavior of ventricular strips in sodium-carbonate solution (equinormal with 0.7 per cent sodium chloride).

When the action of sodium-carbonate solution was first determined, and the results to be described were obtained, it was not known to the writer that this solution had been previously tried, even in combination, upon heart-strips. I have since found, however, that Gaule[1] in an article published in 1878, states that a ventricular strip immersed in sodium-chloride solution made alkaline with sodium carbonate, after exhaustion in sodium-chloride solution, will give a long series of beats. Martius[2] also obtained this result, and offered the explanation that it was due to the fact that the alkaline solution absorbed the carbon dioxide given off by the heart.

Since neither Gaule[1] nor Martius further interpreted this fact, nor investigated the action of sodium-carbonate solution alone fully, I shall describe results obtained with this solution, and conclusions from these at some length, in order to make plain its bearing upon the theory advocated in this paper.

It may be mentioned here that neither Howell[3] nor Lingle,[4] in summing up the ways in which a strip may be made to beat after ex-

[1] Gaule: Archiv für Physiologie, 1878, p. 291.
[2] Martius: Archiv für Physiologie, 1882, p. 543.
[3] Howell: *Loc. cit.*
[4] Lingle: *Loc. cit.*

haustion in sodium chloride, mentions the immersion in an alkaline solution of sodium chloride. Yet this fact has undoubtedly a most important bearing upon their respective theories, which bearing we shall discuss below. Indeed, the importance of the action of sodium-carbonate solutions will be recognized when it is seen that the behavior of a strip in this one solution would make necessary a reconstruction of all our theories as to the action of salts upon heart-muscle, and further give us a most important clue as to the cause of the sodium-chloride arrest.

Upon immersing a fresh strip of turtle's ventricle in a solution of sodium carbonate (equinormal with 0.7 per cent sodium-chloride solution) no series of beats develops. Consider Lingle's theory that sodium ions are the one essential in the origination of heart-beats. Here are sodium ions, yet no beats develop.

Lingle's only reply could be that the sodium-carbonate solution is somewhat toxic, and therefore the sodium ions cannot have their normal effect. Howell might say that beats are prevented in the sodium-carbonate solution because the carbonate ions present precipitate the dissociable calcium as calcium carbonate. Yet both of these possible objections are entirely overcome when we consider the following fact. If after exhaustion in sodium-chloride solution the strip be immersed in sodium-carbonate solution, a series of beats always begins, usually within five minutes, which lasts often for a great length of time, from ten to twenty hours being not infrequent for the duration of this series. I may give here one extract from my note-book to exemplify this interesting result.

May 4, 1904. — At 2.55 P. M. ventricular strips (of the same heart) were immersed in solution of

	Result.
(1) Sodium chloride	Beats in fifty minutes.
(2) Sodium bromide	Beats in thirty-five minutes.
(3) Sodium carbonate	No beats after three hours' immersion.

After leaving strip (2) in the sodium bromide over night and until 10.30 A. M., May 5, it was immersed in sodium-carbonate solution.

Result. — Beats began in three minutes, and lasted over twenty hours.

Let us consider some of the conclusions demanded by the above facts, keeping in mind that the sodium-carbonate solution does not fail to produce beats because toxic, or it would not produce and main-

tain them after exhaustion in sodium chloride, for the same reason it does not fail to produce them because it precipitates calcium.

We come now to face the following facts:

(1) Sodium-chloride solution produces beats in a fresh strip, but cannot maintain them longer than a given period.

(2) Sodium carbonate cannot produce beats in a fresh strip, but can produce and maintain them after previous exhaustion in a sodium-chloride (or bromide) solution.

The action of these two salts is diametrically opposite, yet the cation is the same in both. The only difference between them lies in the different anions, hence the opposite effects can be accounted for only by assuming that the anion plays a most essential part in the action of the salt. We can see no escape from this conclusion.

These facts have a further implication. They must affect our theories as to the cause of the sodium-chloride arrest. The problem of this arrest is one which has not as yet been satisfactorily solved. Loeb has suggested it as due to the poisonous effect of pure sodium solutions. Howell[1] has justly pointed out that there is not sufficient evidence to justify this position. The renewal of beats in a pure sodium-carbonate solution makes this position completely untenable, unless we are to suppose that another anion can neutralize this poisonous effect, which would seem very improbable, and, further, very difficult of demonstration. It might be remarked here that the term poisonous as applied to the action of pure sodium solutions would seem not wholly justified, even if beats could only take place in presence of other cations. Because an animal dies when fed only carbohydrates, we should not be justified in saying that pure carbohydrates are poisonous, and that proteids are needed to neutralize this poisonous effect.

Howell has suggested that the sodium-chloride arrest takes place because nearly all the diffusible calcium has left the strip, yet we know that renewal takes place in solution of lithium chloride, dextrose, hydrogen peroxide, and Ringer's mixture. Three of these contain no calcium ions. Further, as above stated, renewal also takes place in pure sodium-carbonate solutions; this solution not only contains no calcium ions, but, on the contrary, would precipitate these ions as the carbonate, were any present. The theory that the sodium-chloride arrest is primarily due to a lack of calcium would, therefore, not seem supported by the facts.

[1] Howell: This journal, 1901, vi, p. 195.

Lingle,[1] offering another explanation of the sodium-chloride arrest, says: "In this case" (referring to the fact that beats are renewed by oxygen gas after sodium-chloride exhaustion) "recovery occurs without any diffusion of salts, which indicates clearly that the ordinary sodium-chloride arrest is largely due to a lack of oxygen." Later in the same article, Lingle[2] apparently returns to Loeb's position that the poisonous effect of pure sodium solutions is chiefly responsible for the sodium-chloride arrest, and again, in his summary, says that this arrest is due to a lack of oxygen. The reasoning by which Lingle arrives at this conclusion scarcely seems justifiable, for, by exactly analogous reasoning, we should say that this arrest is due to a lack of dextrose, to a lack of calcium chloride, or of any other substance normally found in the blood which will renew beats after the sodium-chloride arrest.

We may be able to come to some satisfactory conclusion regarding this arrest, if we examine it from another point of view. First, we notice that none of the agents which will cause beats after the sodium-chloride exhaustion will cause a series of contractions before this exhaustion. Clearly the muscle must be in a different condition after sodium chloride has acted upon it than before. What difference, we now ask, is most pronounced? The answer is, the tone of the muscle has undergone a distinct change; the recording lever invariably shows a slow continuous loss of tone after immersion in this solution.

We come now to examine the agents which will cause a renewal of the beats after the sodium-chloride exhaustion. Howell[3] sums them up as follows:

1. Immersion in a Ringer's mixture.
2. Immersion in a mixture of sodium chloride and calcium chloride.
3. Immersion in a solution of dextrose or cane-sugar.
4. Immersion in lithium chloride.

To these Lingle adds:

5. Immersion in oxygen gas or addition of hydrogen peroxide to the solution.

We might add here as auxiliary to 5, the method of immersion in moist air. This method was mentioned by Lingle,[4] and corroborated at some length by Martin[5] in 1903.

[1] LINGLE: This journal, 1902, viii, p. 83.
[2] LINGLE: *Ibid.*, p. 97.
[3] HOWELL: *Loc. cit.*
[4] LINGLE: This journal, 1902, viii, p. 83.
[5] MARTIN: *Loc. cit.*, p. 124.

Since these agents differ so widely chemically and physically, some electrolytes, one a non-electrolyte and one a gas, we cannot find the direct cause in the chemical or physical nature of the substance itself. Yet we are able to find a common feature in the action of all these substances upon the heart-strip. This common feature is to cause a more or less marked increase in tone. The rapidity with which a strip recovers in these various agents is more or less directly proportional to the strength and rapidity of its action as a tone-increaser. Sodium carbonate is a sixth agent by which beats may be renewed after exhaustion in sodium chloride. Does this solution also cause an increase of tone in heart-muscle? We answer that a recording lever shows that it does so very rapidly just before the series of contraction begins. Whether the cause of this increase in tone is due to the carbonate ions or the hydroxyl ions, both of which are of course present, has not been determined. In view of Zoethout's [1] work upon skeletal muscle, it seems very likely that the hydroxyl ion is the active agent, since this causes a marked increase of tone in skeletal-muscle. The carbonates have practically the same effect; so that it is very possible that the hydroxyl ion is the cause of the change of tone in both solutions.

When we remember that a loss of tone always occurs before the sodium-chloride exhaustion, and that the various agents which cause a renewal of beats are tone-increasers, it hardly seems possible that this theory of the cause of the sodium-chloride exhaustion is incorrect.

It may be worth while to consider the action of solutions of lithium chloride and dextrose for a moment, since these solutions almost invariably permit of a slight loss of tone before any increase in tone takes place. What might appear at first glance as a weakness in our theory, will, upon consideration, prove to be an additional support to it. Green [2] says that a short irregular series of beats is sometimes obtained in dextrose. This takes place before the dextrose begins to have its effect of increasing the tone of the strip. The anion chlorine is present in large amount in the tissue.

Lingle [3] says that a series of weak beats lasting a short period is sometimes obtained in mixtures of lithium chloride and oxalate. Again this is what we should expect: at first, loss of tone takes place, due possibly to the precipitation of calcium by oxalate ions,

[1] Zoethout: This journal, x, No. viii, p. 373.
[2] Green: *Loc. cit.* [3] Lingle: *Loc. cit.*

and beats begin. Further, in substantiation of our position, we may state that lithium solutions, which are weakest as tone-increasers, are the most difficult in which to get a renewal after sodium-chloride exhaustion; dextrose, which is a little more powerful in this respect, comes next; whereas a mixture of sodium chloride and calcium chloride, which is most powerful as an increaser of tone, causes a most rapid recovery after the sodium-chloride arrest.

Consider whether this theory of the sodium-chloride exhaustion agrees with the history of a strip. In the first place, the operative procedure throws the strip into an increased tone; now only those solutions originate beats in such a strip which permit of a loss of tone (or those which, like calcium, make the muscle exceedingly irritable). Sodium-chloride solution, for example, permits, indeed causes, a loss of tone, and this solution also causes a series of beats. But the action of sodium-chloride solutions is very mild in this respect; hence the long latent period, followed by the long series of beats, which gradually decrease until the tone becomes, so to speak, just below the beating value. If the strip is now changed to a solution which is not toxic, and if the tone be increased by this solution, beats again commence, since the anion chlorine is present in the tissue in large amount. The cation may (as in sodium carbonate) be the same in both solutions, or it may differ (as in calcium-chloride solution).

One objection which may be urged against this position is as follows: calcium-chloride solution causes an increase in tone, yet washing with this solution previous to immersion in sodium-chloride solution usually causes a much shorter latent period. The answer to this objection is, it seems to me, as follows: calcium ions, without doubt, greatly increase the irritability of heart-tissue. Whether they do this simply by causing an increase in tone is yet to be determined. From the fact that calcium will cause an increase in irritability, it is only natural to expect that the strip first washed in it will respond more quickly to whatever stimuli it comes in contact with. It must be remembered that if the calcium be not washed off, or if more than a very small per cent of it be present in the subsequent bathing solution, beats will never develop. The writer's idea is not that the only function of the cation is to maintain tone; it seems very probable indeed that it also has some other functions which will be made the subject of future investigation.

A second objection which may be urged against the above position

is as follows: it sometimes happens that strips after exhaustion in sodium chloride will renew the series of beats in sugar solutions or moist air without visible alteration in tone.

In answer to this objection the following may be stated: First, the strip is in a tone-increasing medium, *i. e.*, one which in most instances increases tone, and it is very possible that a very slight increase in tone has taken place, enough to permit of a series of beats. In the second place, we should remember that the above-cited instances are the exception rather than the rule, and exceptions to the normal behavior of heart-strips are frequent and marked. Thus I have seen a fresh heart-strip give an excellent series of beats (very shortly after immersion) in a solution of potassium iodide equinormal with a 0.7 per cent solution of sodium chloride. This result is very rarely obtained. Further, against this objection, we may state that the *best* series of beats after the sodium-chloride arrest is almost invariably obtained only after an *increase* in tone has taken place.

We have seen thus far that different sodium solutions have different powers of stimulating heart-muscle. It would seem only natural to try solutions containing still other anions to note the effects of each. Among such solutions tried were the following. The solutions used were all equinormal with 0.7 per cent sodium-chloride solution.

The results from the fifteen experiments made with sodium iodide have been very contradictory; a longer series of experiments will be necessary to determine the effect of this salt. There can be no doubt that the iodine ion has an effect produced by neither the chlorine or bromine ion. Thus in about seven of the experiments no beats at all developed in the sodium-iodide solution, though beats did develop normally in controls in sodium chloride or sodium bromide. Further, a good series of beats was obtained upon changing the strips from the iodide solution to a sodium-chloride solution (after two and one-half hours immersion in the former solution) with a very short latent period (again the prime importance of the anion is exhibited). In three experiments made with the sodium-iodide solution a fine series of powerful, regular beats was obtained with a latent period of only eight to ten minutes. The series lasted about one and one-half to two hours. The controls in sodium chloride showed an unusually short latent period also, so that it is probable that these strips were unusually irritable. In the remaining five experiments a series of beats was obtained practically

the same as an ordinary sodium-chloride series. Thus it will be seen that the results obtained so far with sodium iodide are by no means conclusive.

Sodium-nitrate solution usually produces a series very similar to that obtained in sodium chloride. The latent period in the nitrate solution is usually somewhat longer, and the series of beats somewhat shorter than in the sodium-chloride controls. In many cases a short series of beats may be obtained by immersing a strip which has ceased to beat in sodium nitrate into a sodium-chloride solution.

Among other sodium salts tried were the sulphate, acetate, tartrate, and chlorate.

The sulphate gives a good series of beats.

The acetate and tartrate series resemble closely the one obtained with sodium nitrate. In most instances a renewal of beats can be obtained for a longer or shorter period by immersing the strip in sodium chloride or sodium bromide after it has ceased to beat in the other sodium solutions.

But a few experiments have been made with sodium-chlorate solution. The results obtained were, however, very interesting. The latent period was much longer (an hour or more) than in the controls in sodium chloride, while the series of beats which was obtained was very weak and lasted only from one to two hours.

These different results with different solutions can only be explained by the supposition that the anion is of prime importance in the production of heart-rhythm. The results with sodium carbonate alone would seem to the writer conclusive.

Another result of Lingle's [1] may be mentioned which strongly supports the theory herein advocated. He finds that sodium-oxalate solution alone is unable to start beats in ventricular strips, yet a mixture of sodium chloride and sodium oxalate will originate a series of beats which, while not as strong as the series obtained with sodium chloride alone, may continue two hours or more. Lingle appears to have entirely overlooked the importance of his own statement. It strongly supports the theory that it is the anion which is really responsible for the rhythmic activity.

The rhythmic activity of the œsophagus has been said to be analogous in some respects to that of the heart. It may therefore be of interest here to mention some results obtained by Stiles [2] in his

[1] Lingle: This journal, 1902, viii, p. 88.

[2] Stiles: This journal, 1901, v, p. 338.

work with various salt solutions in their action upon the rhythmic activity of the œsophagus.

He found that the œsophagus will not exhibit rhythmic activity in pure sodium-chloride solutions. An excellent rhythmic series is, however, always obtained by treatment with a Ringer's mixture. Among other things he found that there were four salts capable of replacing sodium chloride completely in the Ringer's mixture. These salts were sodium nitrate, sodium bromide, sodium iodide, and sodium chlorate. He also found that some other sodium salts, among which are the tartrate, acetate, butyrate, and lactate, are capable of replacing a certain per cent of sodium chloride in the Ringer's mixture, yet if this substitution be pushed further, no rhythmic contractions were produced by the solution.

These results are directly in line with the theory that it is the anion which stimulates, since different sodium solutions produce very different results.

An objection which may be raised here is that a much greater difference in effects produced by solutions is obtained by changing the cation and keeping the anion the same. We answer that this is exactly what we should expect. Many cations, especially potassium and calcium have marked and immediate effects upon the tone of the muscle. It is a familiar fact that the irritability of a muscle is greatly influenced by its condition of tone; consider, for instance, the example of ordinary skeletal muscle passing into rigor caloris, when stimuli often produce five or six contractions which are entirely unable, under normal conditions, to produce even one.

What then is more natural than that the alteration of the tone of the muscle, by changing the cation, should greatly change, or even entirely alter, its power of reacting to the stimulus furnished by the anion. The fact that some sodium solutions can produce beats, while others, which do not precipitate any cation and are not toxic, are not able to do so, can, we believe, be explained only on the assumption that it is the anion which stimulates. As to the means by which the anion stimulates, no suggestion can yet be offered.

It will be noticed that the theory herein presented coincides (with the exception of the function of the cation) with the one worked out by Mathews[1] concerning the action of salts upon nerve-tissue.

The writer desires to state that he had formulated his theory, and done much of the work upon it, some time prior to the publication of

[1] Mathews: This journal, 1904, xi, No. v.

Dr. Mathews' paper. The fact that very similar conclusions were arrived at in two different fields, by investigators unacquainted with each other's work, would seem to lend additional strength to the theory itself.

In conclusion, it may be said that this paper is intended in the nature of a preliminary communication. While the author regards the evidence offered as necessitating the theory herein advocated, there is much work still to be done upon this problem.

Work is being continued to determine the action of other salts in various strengths upon heart-strips, and to test whether a modification of this theory may be applied to ordinary skeletal muscle.

My sincere thanks are due to Professor Michael F. Guyer, whose continued encouragement and suggestions during this work have been invaluable.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 161.

THE EFFECT OF PIGMENT-MIGRATION ON THE PHOTOTROPISM OF GAMMARUS ANNULATUS S. I. SMITH.

BY GRANT SMITH.

I. INTRODUCTION.

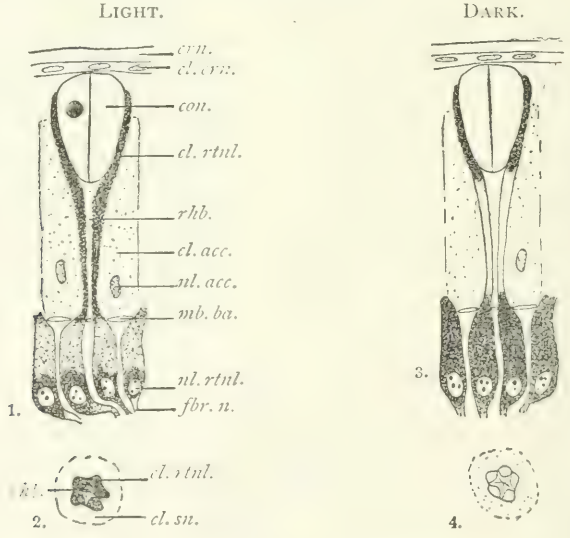
THE migration of the retinal pigment in crustaceans has been known for some time to be a process by which the amount of light reaching the recipient elements can be controlled. Since a number of animals change the sense of their phototropism with a change of the intensity of the light — becoming negative in bright light and positive in dim light — it is conceivable that, in a crustacean, a change in phototropism may accompany the migration of the retinal pigment.

In testing this question I undertook experiments on Gammarus annulatus S. I. Smith, a common salt-water amphipod of the New England coast. The animals were collected at Lynn Beach, Mass., and were kept in the laboratory at Cambridge in sea-water at a temperature of 12° C. In their natural habitat, their actions do not seem to be as rigidly controlled by light as those of some other animals are. As is well known, they are highly thigmotropic and hence may be collected in large numbers from the under sides of loose stones or pieces of board along the seashore at low tide. To the casual observer they would thus appear to be negatively phototropic. But they can also be found abundantly either swimming back and forth across little pools or in gently running water. At such times, when they come to rest, they do not always hide underneath protecting objects, but are very often to be found resting in the bright sunshine. They are in no sense nocturnal; they feed and move about freely in the bright sunlight as though it controlled them no more than it does the higher animals. It should also be recorded,

as having a possible bearing on the question of their responses, that the animals were collected during the spring when they were actively breeding. All the females used in these experiments were either copulating or carrying eggs.

II. Retinal Pigment-Migration.

The retinal pigment-migration in Gammarus annulatus was found to be so like that in G. ornatus as described by Parker ('99, pp. 144–146) that the following statement quoted from the account by him applies equally well to the species at hand.



Ommatidia of Gammarus ornatus after Parker ('99).

Figure 1. — Longitudinal section of an ommatidium, showing the arrangement of pigment due to exposure to bright light.

Figure 2. — Transverse section of a retinula from such a preparation as that shown in Fig. 1, and taken at the level marked *rhb.* in that figure.

Figure 3. — Longitudinal section of an ommatidium, showing the arrangement of pigment due to the absence of light.

Figure 4. — Transverse section of a retinula from such a preparation as that shown in Fig. 3, and taken near the level marked *rhb.* in Fig. 1. Abbreviations: *cl. crn.*, corneal hypodermis; *cl. rtnl.*, retinular cell; *cl. acc.*, accessory pigment-cell; *con.*, cone; *crn.*, corneal cuticula; *fbr. n.*, retinal nerve-fibre; *mb. ba.*, basement membrane; *nl. rtnl.*, nucleus of retinular cell; *nl. acc.*, nucleus of accessory pigment-cell; *rhb.*, rhabdome.

"In an eye of G. ornatus that had been subjected to light for some six hours (Fig. 1), a considerable amount of black pigment

was found uniformly distributed through the distal and middle portions of the retinular cells, thus sheathing the cone and rhabdome laterally (Fig. 2). The proximal portion of each cell contained a few irregularly scattered pigment-granules except near the nucleus, where the pigment was more abundant.

"In an eye from an animal kept some six hours in the dark (Fig. 3), the pigment in the distal portions of the retinular cells presented the same conditions as in the eyes exposed to light. The middle portions, however, were entirely devoid of pigment, while the proximal portions were as densely filled with pigment as the distal portions.

"Obviously the changes induced by the presence or absence of light affect the pigment of only the middle and proximal parts. When an animal that has been kept in the dark is exposed to light, the pigment that is massed in the proximal parts of the retinular cells (Fig. 3) migrates distally and fills the middle portions, without, however, entirely abandoning the proximal parts, especially around the nucleus (Fig. 1). When an animal that has been kept in the light is placed in the dark, the pigment in the middle portions (Fig. 1) migrates into the proximal parts till almost no pigment is left in the middle portions. In other words, the presence of light induces a distal migration of much of the pigment from the proximal parts, and the absence of light brings about a proximal migration of almost all of the pigment in the middle parts."

The rate at which this migration took place was not determined by Parker, and I therefore undertook to ascertain by experiment what it is. Accordingly, live specimens of G. annulatus were placed in the dark-box over night. The next day several of them were poured into a small net and killed immediately by dipping them for a moment into water at 80° C., after which preparations of their eyes were made by further treating, embedding, and cutting them in paraffin. The remaining animals in the dark-box were exposed to the light under the same conditions as those which were subsequently used in the experiments. After they had been subjected to light for fifteen minutes several were removed and killed. Others were treated similarly after thirty minutes, forty-five minutes, and sixty minutes respectively. There were thus at hand for comparison, "dark" eyes, "light" eyes, and eyes in which the pigment was presumably in an intermediate position.

After an exposure to light for fifteen minutes the parts of the

retinular cells free from pigment in the "dark" eye were occupied by many scattered granules, which increased in numbers in the later intervals until the "light" condition of the eye was reached in about an hour. It is noteworthy that the migration of the pigment is very considerable in the first fifteen minutes. No effort was made to learn how soon pigment-migration began, but it would probably be possible to recognize intermediate stages at five and ten minutes after exposure to light. The rate of migration as determined for the eye of G. annulatus agrees fairly well with what Parker ('97) found for the proximal pigment-cells of the more complex eye of Palæmonetes vulgaris. In this species the migration was accomplished in one-half to three-quarters of an hour; while in G. annulatus a full hour is necessary. The way in which the facts of migration bear on the question of the stimulation of the retina can be appreciated from the following quotation from Parker ('99, p. 146):

"The axial light which . . . finds its way into the rhabdome must have a certain degree of intensity in order to stimulate that organ. Ordinary daylight is presumably more than sufficient to call forth this stimulation, and such superfluous light as may pass to the edges of the rhabdome or through it is probably absorbed by the black pigment that in bright light (Figs. 1, 2) surrounds that body. In dim light, however, there must be times when the light which enters the rhabdome is scarcely intense enough to stimulate that organ. Under such circumstances the more oblique rays, which ordinarily would be absorbed by the black pigment on the sides of the rhabdome, would materially aid in stimulating it if they were turned back into the rhabdome. That these rays are probably thus turned back is shown by the fact that in dim light the black pigment is removed from the rhabdome and the surrounding whitish reflecting pigment of the accessory pigment-cells is exposed (compare Figs. 2, 4)."

If this view of the action of the parts of the eye is correct, an animal taken directly from the dark, and with the reflecting layer of the retina exposed, ought to react to light differently from one which had been in the light some time and in which the reflecting layer had consequently become covered. Following a general rule of phototropism, namely, that animals are negative in strong light and positive in weak, we should expect a specimen of G. annulatus taken directly from the dark to be negative, or at least less positive than one which had been some time in the light. To ascertain whether this supposition is true, the following experiments were tried.

III. Phototropism.

1. **Apparatus.** — All the observations on phototropism were carried on in a large dark-room in the Zoölogical Laboratory of Harvard University. The source of light throughout was a large, so-called one-hundred-candle-power, incandescent, electric light. With the exception of an occasional, brief change in the intensity, due to the shifting of machinery at the power-house, this light was steady and reliable. Moreover its intensity could be accurately measured, a matter of great importance, in studies in phototropism which aim to be accurate. By the use of a Lummer-Brodhun photometer and a standardized candle it was ascertained that the intensity of this light after a heat-screen had been placed in front of it was 52.7 candle-power. The lamp was enclosed in a black sheet-iron chamber open on one side. The heat-screen consisted of a flat, rectangular glass jar filled with water, which was changed from time to time as it became warmed.

The aquarium in which the animals were tested was about four inches deep, twelve inches wide, and twenty-four inches long. Inside it was painted a dead-black, in order to prevent internal reflection. The two ends were made of glass. On the inside of the end away from the light, which may be called the negative end, was fitted a black-painted slate to absorb the light. An aquarium of relatively large size was used, because G. annulatus is very active and not pronouncedly phototropic; hence the larger the aquarium, the better the chance of discovering a tendency for the animals to remain near or away from the lighted end. Furthermore, thigmotropic responses to the sides of the aquarium being less frequent if the tank is large, one of the most disturbing influences in such experiments may be reduced by this means. In order to cut off reflections from the walls of the room, the aquarium was placed in a frame-work which was hung round with black cloth, within which light was admitted only through the water of the aquarium. The central point of the aquarium was placed 1 metre from the source of light. Under these conditions the maximum intensity of light at the positive end of the aquarium was about 110 candle-metres. The intensity of light at the negative end varied from 30 candle-metres, when very clear water was used, to something below this intensity, if, as sometimes happened, the water was slightly turbid. The animals chosen for a given day's experiments were placed the night before in a dark-box

in the dark-room where the water had a temperature of 15° C., the same as the water in the aquarium. The water was aerated artificially by passing bubbles through it.

2. **Experiments.** — Many preliminary observations were made upon single individuals in order to discover the general tendency of their responses, and greater accuracy was obtained as errors in the method of treatment were eliminated. It is especially important that the water in which the animals are swimming be free from débris, otherwise thigmotropism may overbalance the responses to light. The animals should be transferred to the middle of the aquarium in the dark and allowed to remain quiet for two or three minutes before the light is turned on. It was not noticed that the phototropic response was changed by handling the animals, as Towle (:00, p. 351) found for Cypridopsis. But it was observed that the animals would sometimes scurry to the bottom upon being transferred to the aquarium, and lie there quietly for a time, just as they do when one attempts to capture them at the shore. Now and then animals were found which were not typically active, and such were discarded. Occasionally individuals were found which gave responses opposite to those of the majority. This lack of homogeneity was probably due to the fact that the specimens were not brought to the laboratory as a single colony, but in several small catches, and consequently under somewhat different physiological conditions. Inasmuch as Parker (:02, p. 110) found that the responses of male and female copepods are not alike, the amphipods were separated on the basis of sex. Most of the observations were made on females, though as a matter of fact there proved to be little, if any, difference in the phototropic reactions of the two sexes.

My method was to use several animals at a time, and to record at half-minute intervals the number to be found in the positive half of the aquarium (*i. e.*, the half toward the light) during an hour. To use more individuals than one can count at a glance renders accurate observations impossible, and hence six animals were chosen as the largest number that could be easily and instantly counted. In Table I, which illustrates the method of keeping the records, and is the record for Set 4 in Table II, each number in the horizontal rows in the body of the table represents the number of individuals, out of six, which were found in the positive half of the aquarium at the end of given half-minute intervals. For convenience, these are grouped into ten-minute periods, each one of which is represented by a horizontal

row of figures, and at the end of each of these rows a total is given, which represents the number of records for the positive half in a possible total of 120. The table contains records of six ten-minute intervals of the first hour, and a final record (seventh ten-minute period) taken at the beginning of the second hour.

TABLE I.

Records of the number of individuals, out of six, found in the positive half of the aquarium at the end of half-minute intervals during an hour and ten minutes.

10-minute periods.	Number of individuals in positive half of aquarium at end of half-minute intervals in each 10-minute period.																			Totals.	Differences between + and − halves of aquarium in		
																					Nos.	Per cent.	
1st	2	2	4	1	3	2	4	2	2	2	3	3	3	4	1	4	5	2	4	5	58	4	−03.3+
2d	3	4	4	4	4	5	4	5	3	5	5	5	3	4	4	6	4	5	4	5	86	52	43.3+
3d	5	4	6	5	5	4	5	3	5	4	6	4	3	5	2	3	5	5	5	5	89	58	48.3+
4th	5	3	5	6	4	4	4	5	5	5	5	5	5	3	3	3	5	5	4	4	88	56	46.6+
5th	6	6	6	6	6	4	5	5	4	6	4	6	5	5	4	4	5	5	4	4	100	80	66.6+
6th	5	3	5	4	4	5	5	6	4	5	4	5	6	5	6	4	5	5	5	5	96	72	60.0
7th	6	6	6	5	5	4	5	5	6	3	6	4	4	4	6	5	3	5	6	6	100	80	66.6+

From an inspection of Table 1 it is to be seen that the positive half of the aquarium was not entirely free from animals at any time during the entire seventy minutes, at the instants when the records were taken. There were only two occasions when the number in the positive end was reduced to one, and these occurred during the first ten minutes. On the other hand, the negative half was found entirely deserted twenty-two times, but all these observations were made some time after the first ten minutes. Moreover, during the first ten minutes the animals were so distributed that the total of positive records, 58, was very near to the indifferent point, 60; in the second ten minutes this total mounted quickly to 86, after which it showed a fluctuating increase but never reached the possible maximum of 120.

It must not be supposed that the totals recorded in Table I represent the real influence of the light, nor is it easy to get an exact expression for this. But it seems probable, since the animals while in

the dark coursed more or less continuously from the positive to the negative end of the aquarium and back again, that some of this locomotion takes place in the light. In the case of a positive animal, a measure of this activity can be found in the number of times animals are present in the negative half of the aquarium; and if this number is subtracted from the number of records for the positive half, the remainder should be a measure of the true light-reaction. This computation has been carried out for Table I, and is given in the last two vertical columns in that table. From this, as well as from other aspects of Table I already alluded to, it appears that the animals were indifferent or slightly negative in their responses at first, and that they afterward became quickly and decidedly, though not completely positive.

Seven other sets of animals were dealt with in the way just described, and a summarized statement of the results, including those from Table I, is given in Table II.

TABLE II.

Summary of the records on eight sets of observations each similar to the one given in detail in Table I.

10-min. periods.	Sets of observations.								Totals.	Averages.	Per cent.	Differences between + and − halves of aquarium in	
	1.	2.	3.	4.	5.	6.	7.	8.				Nos.	Per cent.
1st	87	96	80	58	57	59	88	87	612	76.5	63.7	264	27.5
2d	101	97	91	86	71	74	99	100	719	89.8	74.8	478	49.8−
3d	95	101	94	89	71	85	89	99	723	90.3	75.2	486	50 6+
4th	100	97	98	88	75	93	95	94	740	92.5	75.4	520	54.2−
5th	110	98	88	100	65	83	100	97	741	92.6	75.5	522	54.4−
6th	101	106	97	96	76	63	100	108	747	93.3	76.1	534	55.6+
Retested after			10 min. 95	0 min. 100	70 min. 87	20 min. 92	170 min 104	25 min. 101	579	96.5	80.4	438	60.8+

In comparing this table with Table I we see in Sets 5 and 6, as in Set 4 just described, that the responses during the first ten-minute period are slightly below the indifferent point, *i. e.*, the animals are

slightly negative. This uniformity is probably due to the fact that the animals for these three experiments were brought from the shore in one lot, and thus probably were in a uniform physiological condition. In the five other sets (Table II) the response was not negative at any time, but *it was much lower at first than subsequently*.

There is the possibility that the use of a stronger light would have rendered even these five remaining sets of animals negative at first; and thus the table would have shown a generally negative condition at the outset. However this may be, it is certain that the light-responses in three of my sets out of eight were for a few minutes slightly negative, and in all the sets the responses were not strongly positive at first. Indeed, if we examine Table I more closely we shall find that the animals were more strongly negative in the first five minutes, than the total would indicate. During that period the possible maximum number of responses was sixty, and of this number only twenty-four were positive, while thirty-six were negative. Hence the difference, twelve negative responses, is a measure of the true light-influence for the first five minutes. The results were similar in Sets 5 and 6 (Table II). Thus we probably have in these records evidence of the well-known phenomenon of reversal of phototropism. But it differs from all other cases, I believe, in the fact that the reversal was quickly made and without any increase of light at its source.

If the responses for the first ten minutes in Sets 4, 5, and 6 be left out of consideration, the remaining responses are uniformly positive. This was quite the reverse of what was to be expected from the general statement made by Holmes (:01, p. 212), that the aquatic amphipods are negative. The specimens of G. annulatus (= G. locusto Linn.[1]) which Holmes used were from a fresh-water pond at Falmouth, Mass., and the light and other conditions under which he experimented were so different from those in the present case that a basis for the accurate comparison of results is wanting. Holmes has shown that the phototropism of amphipods was at times reversed by foul water. It did not seem possible that I had used foul water in my experiments, but to exclude this point with certainty, I made tests with animals and water brought fresh from the shore. These animals also were not negative, though they were by no means as

[1] Dr. HOLMES informs me that since the publication of his paper he has determined that the species with which he worked was G. annulatus rather than G. locusto Linn.

active as the previous lots. I think there is no doubt that under the conditions of my experiments G. annulatus, though indifferent or slightly negative at the outset, is generally positive. This is, of course, particularly true after the animals have remained for some time in the light. In Table I it has been shown that the animals were strongly positive after they had remained in the light for an hour, giving a total of 100 positive reactions out of a possible 120, and with a light-response of 80 (67 per cent). The average of the last (seventh) series of records in Sets 3 to 8 (Table II), though the observations were not all made at exactly the end of an hour, also shows essentially the same condition, in that there are 579 positive reactions out of a possible 720, and therefore a light-response of 438 (60 per cent). Though usually positively phototropic, G. annulatus is certainly not so remarkable an example of this as Talorchestia longicornis, which, according to Holmes (:01, p. 213), follows a light carried about in a darkened room, and remains positive even to light so intense as to cause its death. In the present case, however, it is significant that not one of the nine hundred and sixty half-minute records for the eight sets showed the positive half of the aquarium entirely deserted; and an average of 4.5 out of a possible six animals were in the positive half all the time. On the other hand, the negative half was frequently free from animals.

Connected with the reversal in the direction of the response already mentioned, is the interesting fact that the positive response is not constant, but gradually increased for an hour. In Table II, it may be seen that the response starts low in every set, even if it is not negative, and that in every set there is a sharp increase of the positiveness during the second ten minutes as compared with the first, after which the rise is more gradual to the end of the hour and somewhat beyond. There are two points in Table II at which the responses decreased markedly: in the fifth period of Set 5 and in the sixth period of Set 6. Since, however, the responses rise immediately after each of these to a higher level, it is probable that these depressions are purely accidental. Notwithstanding the fluctuations, it must be evident that when the animals first emerge from the darkness they are individually indifferent, slightly negative, or more frequently slightly positive to light; that within ten minutes they change and become decidedly positive, — a condition that is gradually intensified for an hour or more.

IV. Discussion of Results.

From what has been said in the preceding sections it is plain that the distal migration of the retinal pigment in G. annulatus is accompanied with a change in the animal's phototropism. During the first fifteen minutes of exposure to light, there is a decided movement of retinal pigment distad, and parallel with this there is a very rapid rise in the number of positive responses. Then follows a period of some three-quarters of an hour, during which a more gradual pigment-migration distad is accompanied by a gradual increase in positive responses. Thus it appears that with an increased protection of the rhabdome from light by the migration of pigment there goes an increase in positive phototropism.

The effect of retinal pigment-migration on phototropism is so evident from these observations that clearly it must be taken into account in the study of the photic reactions of any animal whose eyes show this phenomenon. It has been shown that an animal may respond to a sudden change in the intensity of illumination by a gradual alteration in its reactions, and that this alteration is dependent upon the adjustment of the retinal pigment to the new conditions. In studying the phototropism of such an animal, it is therefore necessary to know what was the position of the pigment when the experiment began, what length of time it requires to reach the extreme conditions, what the previous illumination had been, and what intensity of light is being used. To institute just comparisons it is desirable that investigators should have some common starting-point and in work of this kind it is obvious that the "dark" condition is the only basis from which experiments may safely proceed.

It is my hope soon to contribute something more in reply to some of the questions not answered by these observations. My warmest thanks are due to Professor G. H. Parker, whose supervision I have had in these experiments.

V. Summary.

1. In Gammarus annulatus, as had previously been shown by Parker in G. ornatus, the retinal pigment in the "dark" eye is accumulated in the distal and the proximal ends of the retinular cells and leaves the rhabdome exposed to the reflecting action of the accessory pigment. In the "light" eye the rhabdome is ensheathed

by the retinal pigment, and the accessory pigment can no longer act as a reflector.

2. The migration of the pigment from the condition in the "dark" eye to that in the "light" eye is rapid during the first fifteen minutes of exposure to light, and then slower till its completion, which occurs in about an hour.

3. G. annulatus when taken from the dark and exposed to a constant light of 30 to 110 candle-metres in intensity is, during the first ten minutes, either indifferent, slightly negatively or slightly positively phototropic. This condition rapidly gives way to a strongly positive phototropism, which gradually increases, in the course of an hour or so, to a maximum.

4. The retinal pigment-migration, since it controls the amount of light reaching the rhabdomes, is in all probability the means of inducing this change in phototropism.

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THE NATURE OF CARDIAC INHIBITION WITH SPECIAL REFERENCE TO THE HEART OF LIMULUS.

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1. The Interpretations of the Action of the Vagus on the Heart.

BUT for the fact that the unique anatomical relations between the nervous and the muscular tissues in the heart of Limulus render it possible to determine whether the inhibitory nerves act on the ganglion or directly on the heart-muscle, or on both, the subject of inhibition of the Limulus heart might be dismissed with the statement that the heart is connected with the brain by two pairs of inhibitory nerves, the stimulation of which produces the identical phenomena observed in the vertebrate heart on stimulation of the vagi. The view originally advanced by Weber that the vagi inhibit the automatic activity of the ganglion cells in the heart in a manner analogous to the processes of inhibition in the central nervous system has been replaced by the theory that the cardio-inhibitory nerves act directly on the heart-muscle. The latter theory, based mainly on the researches of Gaskell and Engelmann and their students, is very generally accepted. It is a necessary deduction from the myogenic theory of the heart-beat and the nature of co-ordination or conduction in the heart. If the heart-rhythm is an expression of the metabolism of the heart-muscle, or due to chemical stimulation from the blood, and not the result of nervous impulses reaching the muscle from automatic or reflex nerve-centres, it is self-evident that any force that shall stop that rhythm must act directly on the muscle. The two theories can thus not part company. When conclusive proofs of the myogenic nature of the rhythm and the conduction are forthcoming, the other follows as a matter of course. Apart from the observations which appear to count in favor of the myogenic theory, such as the rhythm of the embryonic heart, the view that the inhibitory nerves act directly on the heart-muscle seems to

receive support in the nature of the changes produced in the heart by the stimulation of the nerves. The inhibitory nerves diminish the rate and the strength of the beats, the power of conduction, and the excitability to direct stimulation. Weber himself observed that the strength of the heart-beat may be diminished by stimulation of the vagi. Now, in as much as the heart-muscle contracts according to the "all-or-none" law, this diminution of the amplitude of the beats is, according to Gaskell's reasoning, a conclusive proof that the vagi act directly on the heart-muscle in a manner to diminish the power of contraction. If the premise were sound, this conclusion would be valid. That the excitability of the heart to direct stimulation is diminished — some have even claimed abolished — during stimulation of the vagi was first pointed out by Schiff. The influence of the inhibitory nerves on the conductivity in the heart has been particularly elucidated by the researches of Gaskell and Engelmann.

While the above interpretations of the nature of the action of the vagi on the heart are corollaries of the myogenic theory of the contraction and the conduction, and must be accepted as proved as soon as conclusive demonstration of the myogenesis of the normal beat and the normal co-ordination is forthcoming, it is evident that all of these conclusions do not necessarily fall to the ground with the myogenic theory. How can the influence of the inhibitory nerves on the heart be reconciled with the neurogenic theory? The sum-total of the numerous researches on the nature of cardiac inhibition since the time of Weber is this, that the inhibitory nerves decrease the rate and the strength of the beats, the power of conduction, and the excitability to direct stimulation; but whether this action of the nerves is on the intrinsic nervous tissue, or on the muscular tissue, is, it seems to me, just as much an open question to-day as it was fifty years ago. The view that the inhibitory nerves diminish conductivity in the heart by direct action on the muscle is incompatible with the neurogenic theory, for if the conduction and co-ordination take place in the nervous elements, as is the case in the Limulus heart, the inhibitory nerves can diminish the rate and the power of conduction only by acting on these nervous elements. But if the contractions are caused by nervous impulses from the ganglion-cells, the inhibitory nerves may retard and diminish or stop these contractions, either by acting on the ganglia in a way to stop or diminish their activity, or by acting on the heart-muscle in a way to render it less excitable to the nervous impulses reaching it. But does not the

fact that the inhibitory nerves diminish the excitability of the heart to direct stimulation, and diminish the amplitude of the beats, prove conclusively that they act directly on the muscle? That the heart of some of the higher vertebrates responds to direct stimulation in accordance with the "all-or-none" principle, appears to be well-established; but that the heart-muscle, apart from the nervous complex, responds to stimuli in this manner is an assumption with scant experimental evidence in its support. We know as yet of no drug or solution that abolishes the action of the accelerator or intrinsic cardiomotor nerves on the heart without injury to the muscle.[1] Hering has lately shown that the augmentor nervous mechanism in mammals (rabbits, dogs, cats, monkeys) lives on for many hours after the death of the animal, and may be restored by Ringer's solution, even after freezing the heart and the heart-nerves for hours.[2] Since we have not yet succeeded in throwing out the augmentor or motor nervous mechanism in the heart, we do not yet know the properties of the heart-muscle apart from this nervous mechanism, and it is furthermore evident that the diminished excitability of the heart during stimulation of the vagi is no proof that the vagus fibres act on the heart-muscle. At least to some kinds of stimuli ganglion-cells have a higher excitability than nerve-fibres, and nerve-fibres a higher excitability than muscle-tissue. A diminished excitability of the nervous elements in the heart, unattended by any change in the excitability of the muscle itself, would, therefore, in all likelihood diminish the excitability of the heart to direct stimulation. This action of the inhibitory nerves is, therefore, as readily explained on the neurogenic as on the myogenic theory.

The changes in the electrical tension of the heart (ventricle) of the toad on stimulation of the vagi is also ascribed by Gaskell, who discovered the fact, to a direct action of the inhibitory fibres on the muscle.[3] The point of peculiar interest is this, that the change in electrical tension on stimulating the vagi is the opposite of that caused by stimulation of the augmentor-nerves. But there is nothing in Gaskell's experiments which would warrant us ascribing these changes in the electrical tension to changes in the muscle rather than to changes in the nervous tissue. If the inhibitory and the augmen-

[1] HERING: Archiv für die gesammte Physiologie, 1903, xcix, p. 250; CARLSON: Science, 1904, xx, p. 684.

[2] HERING: Archiv für die gesammte Physiologie, 1903, xcix, p. 245.

[3] GASKELL: Journal of physiology, 1887, viii, p. 404.

tor nerves induce changes of opposite electrical sign in the ganglion cells or the cells and their processes as well, under the conditions of Gaskell's experiment this variation in tension would be registered by the electrometer just as readily as a corresponding change in the heart-muscle. It would, therefore, seem that the changes in the heart produced by the stimulation of the cardio-inhibitory nerves can just as readily be interpreted on the neurogenic as on the myogenic theory. And as far as the vertebrates are concerned, we have no conclusive proof in favor of either interpretation, just as we have no clear-cut demonstration of the myogenic or the neurogenic nature of the contraction,[1] although the action of some of the drugs on the heart can with difficulty be interpreted on the Gaskell-Engelmann hypothesis.[2]

It has been shown in previous papers[3] that in Limulus the cause of the heart-beat is to be sought in the ganglion-cells, and that conduction in the heart takes place in the nervous and not in the muscular tissue. Of these two facts there can be no question, as their demonstration is direct and relatively simple. These facts alone

[1] PORTER (Journal of experimental medicine, 1897, ii, p. 391; American textbook of physiology, i, p. 151) regards his observations on the ventricle of the dog as a demonstration of the myogenesis of the heart-beat. The fact that the isolated portion of the ventricular apex continued to beat with a rhythm different from the rest of the heart seems to exclude the possibility that the contractions were caused by impulses through nerve-fibres that might have remained intact along the course of the nutrient artery. Isolated portions from any region of the ventricle of the hagfish continue to beat rhythmically for hours, even in the absence of nutrition; but nothing can be concluded from this, as nothing is at present known of the nervous elements in the heart of this animal. It is well known that the mammalian ventricle possesses greater degree of automatism than does the ventricle of reptiles and amphibians; but even if it is a fact that an isolated portion of the apex of the dog's ventricle continues to beat for some time when fed with blood through its nutrient artery, that does not prove that the heart-rhythm is myogenic, because the question of the presence of nerve-cells in the apex of the ventricle can not be considered as settled in the negative. SCHWARZ (Archiv für mikroskopische Anatomie, 1899, liii, p. 63) and others have described a multitude of cells along the course of the nerves and the arteries over the entire ventricular myocard; but SCHWARZ does not think that these cells are nervous in their nature, chiefly because of their small size and the absence of nucleated envelopes. It might be asked why all the small nerve-cells in the heart should be provided with a nucleated envelope, when this is not the case, for example, in the plexuses of AUERBACH and MEISSNER.

[2] HARNACK: Archiv für Physiologie, 1904, p. 415.

[3] CARLSON: This journal, 1904, xii, p. 67; *Ibid.*, 1905, xii, p. 471.

make Weber's theory of the mechanism of cardiac inhibition a possibility, if not a probability. We will now proceed to show that Weber's theory is not only a possibility, but an actual fact, at least in the Limulus heart.

2. The Anatomy of the Cardio-regulative Nerves in Limulus.

For a more detailed account of the cardiac nervous system of Limulus, the reader is referred to the papers of Milne-Edwards,[1] and Patten and Redenbaugh.[2] The intrinsic nervous system of the heart consists, in short, of a ganglionated cord running the whole length of the heart in the dorso-median line, and a system of nerves passing from either side of this nerve-cord to the heart-muscle, the several nerves uniting in one common nerve-trunk at either lateral angle of the heart. This intrinsic nervous system is connected with the central nervous system by an elaborate complex of nerves shown in Fig. 1. These nerves have been worked out in great detail by Patten and

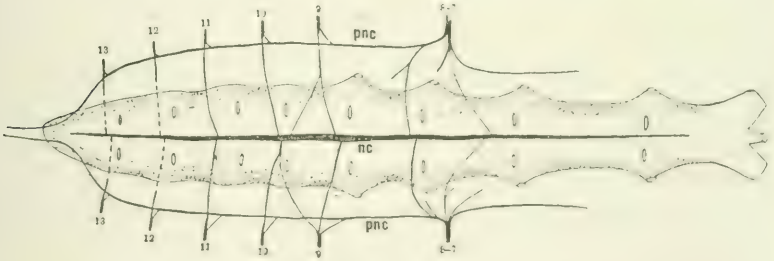


Figure 1. — Heart of Limulus, dorsal view. *n. c.*, dorso-median nerve-cord. *p. n. c.* pericardial nerves. 7–8, cardiac branch from the last two hæmal nerves from the brain. 9–13, cardiac branches from the hæmal nerves of the abdominal ganglia. 7–8, contain inhibitory, 9–11, augmentor fibres.

Redenbaugh, and the only point touching the anatomy that I have to add to the excellent figures and descriptions of these authors is the fact that the two posterior pairs of nerves from the dorsal side of the brain make connections with the nerve-cord on the heart in the manner shown in the diagram. Patten and Redenbaugh were not able to trace these nerves to the heart-ganglion. It can be done, however, in the largest specimens, and stimulation of the nerves near the brain

[1] Milne-Edwards: Annales des sciences naturelles, 1873, sér. 5, xvii.
[2] Patten and Redenbaugh: Journal of morphology, 1899, xvi, p. 91.

shows by its effects on the heart that the relations disclosed by the dissection are not due to mistaking connective tissue-fibres for nerve-fibres.

From the dorsal side of the brain, corresponding to each pair of nerves to the ambulatory appendages, is given off a pair of relatively tiny nerves which take a dorsal direction to innervate the integument, the viscera, and the dorsal musculatures. These nerves are evidently homologous to the nerves occupying a similar relation with reference to the thoracic ganglion in the crayfish (Palinurus). The anterior pair of nerves from each abdominal ganglia takes a similar course and makes connections similar to those made by the nerves from the dorsal side of the brain. As these nerves go to innervate structures dorsal or hæmal to the level of the central nervous system, Patten and Redenbaugh call them "hæmal" nerves in contradistinction from the nerves to the ambulatory appendages and the gills, which they designate the "neural" nerves.

The hæmal nerves from the abdominal chain of ganglia take a dorsal and posterior direction, and, after giving off branches to the digestive tract and the integument, penetrate the pericardial cavity, sending small filaments in the dorsal pericardium to unite with the median nerve-cord on the heart approximately opposite the fourth to the eighth pairs of ostia. These cardiac branches, with the exception of those from the ninth and tenth pairs of nerves, are so tiny that they cannot readily be distinguished from the connective tissue-fibres in the pericardium. Before the nerves enter the dorsal pericardium to connect with the nerve-cord, each nerve sends a communicating branch to the relatively large nerve-trunk which runs parallel to the heart at either angle of the pericardial cavity. These nerves are termed pericardial nerves by Patten and Redenbaugh.

Of the hæmal nerves taking their origin from the brain, the only ones I was able to trace to the heart were the last two pairs (7, 8). The cardiac branches of these two nerves unite in one common trunk before reaching the pericardial cavity. The main branches of this nerve go to make up the pericardial nerve and to supply the large inter-tergal muscle, which lies dorsal to the heart in this region. The branches that pass to the epidermis dorsal to the heart connect with the ganglion on the heart in the manner represented in Fig. 1. There is considerable individual variations as to the exact place of union with the nerve-cord. In some specimens there appears to be connections only at the level of the second pair of ostia, in others the con-

nections are made in the middle of the third segment, while in still others the main if not the only connections are the ones just behind the third pair of ostia.

3. The Physiology of the Cardio-Regulative Nerves.

In order to study the influence of the brain and the abdominal ganglia on the heart, it is not practicable to expose the heart from the dorsal side by removing the carapace dorsal to the heart, because this can rarely be done without some injury to the nerves in the dorsal pericardium. The dorsal carapace may be removed in the region of the first heart-segment, so as to allow the movements of the heart to be observed, or the anterior end of the heart may be exposed from the ventral side by removing the intestine and the reproductive gland. This can be accomplished without any injury to the nervous connections with the heart. For more accurate work it is desirable to connect the heart with a recording apparatus. The following proved to be the only feasible method. After exposing the brain and the abdominal ganglia from the ventral side, the carapace was severed transversely in the region of the first pair of cardiac ostia, and the anterior portion of the carapace removed, care being taken in removing the digestive tract, the reproductive gland, and the dorsal muscles not to injure the exposed heart-segment. The remaining part of the animal was now tilted edgewise on the supporting platform, and held by clamps in such a way that one side of the exposed heart-segment could be connected with the recording lever, the opposite side of the heart-segment being held in a fixed position by means of a clamp. The force of the contraction of the heart is in the transverse direction, owing to the transverse arrangement of the muscle-fibres. In fact, when the heart is severed from the suspensory ligaments it actually elongates on contraction. The above contrivance thus allows graphic records to be obtained of the influence of the ventral nervous system on the heart. But there is this difficulty to contend with, that the hæmal nerves send fibres not only to the heart and the integument (sensory), but also to the inter-tergal muscles and other muscles about the pericardial cavity. Stimulation of the brain, the abdominal ganglia, or the hæmal nerves directly produces contractions of these muscles, which in turn alters the tension on the heart and displaces it to some extent, thus obscuring the tracings. The errors from this source can be eliminated by stimulating the cardiac branches of the hæmal nerves in the pericardium.

As long as the hæmal nerves are intact *stimulation of the brain with the weak interrupted current inhibits the cardiac rhythm.* The tracing in Fig. 2 is typical of these inhibitory effects. Complete inhibition

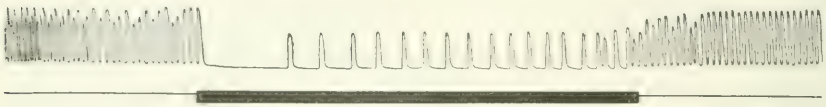


FIGURE 2. — Record from anterior end of heart on stimulation of the brain, the hæmal nerves being intact. Incomplete inhibition. Weak interrupted current.

of the heart is not maintained in any case for more than a few minutes, but on continuing the stimulation, a diminution of the rate and the amplitude of the beats may be observed for as long as twenty minutes. Mechanical stimulation of the brain also produces complete or incomplete standstill of the heart. And these inhibitory effects are furthermore produced by stimulation of any of the sensory nerves connected with the brain. Stimulation of the central end of any of the nerves to the ambulatory appendages with the weak interrupted current diminishes the rate and the strength of the beats, at least at the beginning of the stimulation. This appears to be a true reflex inhibition, and any of the sensory nerves to the brain appears to be able to act as a " depressor " to the heart-rhythm. The inhibition of the heart on stimulation of the afferent nerves to the brain is neither as marked nor as long maintained as that produced by stimulating the brain directly. A typical tracing showing the reflex inhibition is reproduced in Fig. 3.

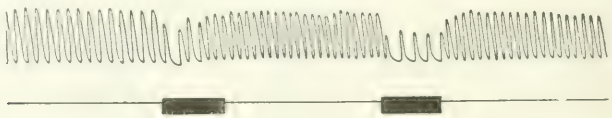


FIGURE 3. — Reflex inhibition of the heart by stimulating one of the ambulacral nerves with a weak interrupted current. Hæmal nerves from the brain intact.

It can be shown by direct experiment, as well as by the process of elimination, that the inhibitory fibres to the heart leave the brain in the last two pairs of hæmal nerves. After cross-section of the abdominal commissures near the brain, direct stimulation of the brain or stimulation of the ambulatory nerves still causes inhibition. Nor does lesion of the hæmal nerves 1 to 6, that is, all the hæmal nerves save the last two pairs, abolish the inhibitory effects of stimulation

of the brain, but after lesion of the last two pairs (7, 8), leaving all the other hæmal nerves intact, stimulation of the brain has no effect on the heart, that is, in case the abdominal commissures are also severed. When the hæmal nerves 7 and 8 are isolated and stimulated near the brain, typical cardiac inhibition is produced. There appears to be no difference in the nerves on the right and those on the left side in their efficacy to inhibit the heart, but the inhibition is usually more complete, and may be maintained for a longer time on stimulation of the seventh than on stimulating the eighth pair of nerves. It is, therefore, probable that the majority of the inhibitory fibres pass to the heart in the seventh pair of hæmal nerves. The tracing reproduced in Fig. 4 is typical of the effects on the heart of stimulating the hæmal nerves 7 and 8 near their point of union with the brain.

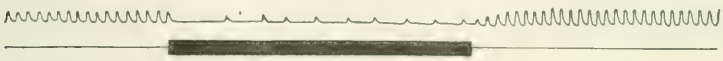


FIGURE 4. — Record showing inhibition of the heart on stimulation of the hæmal nerves 7 and 8 near the brain.

The sixth pair of hæmal nerves sends branches to the tegmentum and the connective and epithelial tissues that go to form the dorsal pericardium in the anterior region of the heart. On the analogy of the cardiac branches of the other hæmal nerves, branches from the sixth pair ought to connect with the nerve-cord on the dorsal side of the heart at the level of the first pair of ostia, but Patten and Redenbaugh were unable to find such a connection, and I have been equally unsuccessful in the attempt. It is certain that stimulation of this sixth pair of nerves produces no effect on the heart, either in the line of inhibition or augmentation, and the same is true of the hæmal nerves given off from the brain anterior to this pair of nerves.

If the abdominal commissures are left in connection with the brain, stimulation of the brain after severance of the inhibitory nerves augments the heart-action This augmentation is also produced on stimulating the commissures directly, as well as by stimulating the first three abdominal ganglia. The augmentation is usually most marked on stimulating the commissures at any point between the brain and the first abdominal ganglion. The cause of this difference is probably this, that on stimulating the commissures at any point between the brain and the first abdominal ganglion, all the augmentor-fibres passing from the different abdominal ganglia to the heart are

affected, while on stimulation of each ganglion, or each pair of hæmal nerves separately, only a few of the augmentor-fibres are being stimulated at any one time. The latent period of the augmentation ranges from five to eight seconds. The latent period of the inhibition is less than a second. The relation is thus identical with that in the vertebrate heart. The accelerator-effects outlast the period of stimulation considerably, which is not the case with the inhibitory effects produced by stimulating the brain or the inhibitory nerves near the brain. If the rate of the heart-beat is relatively low, or from ten to sixteen per minute, stimulation of the commissures usually augments the rate from twenty-five to thirty, but if the rhythm, through previous stimulations or other causes, already approaches those figures, the stimulation of the commissures may not produce a further quickening of the beats, although the amplitude of the contractions is increased (Fig. 5). In a few instances the stimulation of the commissures

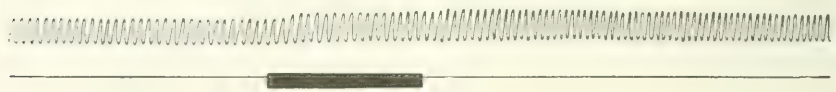


FIGURE 5. — Tracing showing augmentation of the heart-rhythm by stimulation of the abdominal commissures between the brain and the first pair of abdominal ganglia. The commissures severed from the brain.

produced a slight inhibitory effect on the heart prior to the augmentation. This probably means that in some individuals a few inhibitory fibres enter the heart through the abdominal commissures and the hæmal nerves from the abdominal ganglia. Some of the accelerator-fibres may also pass to the heart in the hæmal nerves 7 and 8, but in these nerves the inhibitory fibres are so preponderant that the influence of a few augmentor-fibres, if present, would be obscured. There are no augmentor after-effects on stimulation of the inhibitory nerves. We may therefore conclude that *the inhibitory nerves reach the heart in the two last pairs of hæmal nerves given off from the brain, and the augmentor-nerves reach the heart along the hæmal nerves from the abdominal ganglia.*

Having once determined the course of the inhibitory and the augmentor-fibres from the central nervous system to the heart, the further study of the action of these nerves on the heart was made by stimulating the nerves in the pericardium. This was done partly to eliminate possible errors from the changes in tension and pressure on the heart by contraction of muscles adjacent to the heart on stimulation

of the hæmal nerves near their central origin. The graphic method can also be employed with greater accuracy when the nerves are isolated and stimulated in the pericardium, for in that case the heart may be removed from the body and connected with the recording lever in the ordinary way by hooks in the lateral arteries. The cardiac branches of the hæmal nerves from the abdominal ganglia (9–13) are very tiny and therefore not readily isolated in the living tissue for the purpose of experimentation. In the region where these nerves enter the pericardium, the latter comes into close contact with the carapace, hence the removal of the carapace is liable to injure or sever some of them. These facts probably account for the usual failure to perceptibly influence the cardiac rhythm by stimulating these nerves in the pericardium. But when the stimulation proved effective, the results were augmentation of the rhythm, or the same effects as that produced by stimulation of the commissures or the abdominal ganglia directly.

The inhibitory nerves are more easily isolated in the pericardium for the purpose of experimentation, although they branch considerably in the epithelium and connective tissue before making connections with the nerve-cord on the heart. The typical effects of stimulating these inhibitory nerves near their entrance into the nerve-cord is shown in Fig. 6. The rate and the amplitude of the beats are de-



FIGURE 6. — Record from the sixth segment of the heart. Inhibition by stimulation of the inhibitory nerves in the pericardium.

creased during the stimulation. The diminution in the rate may be relatively greater than the decrease in amplitude or *vice versa*, but I never succeeded in obtaining a diminution of the rate with the strength of the beats remaining unaltered, or a decrease in the amplitude of the contractions not accompanied by a slower rhythm. Complete inhibition of the heart is less frequently obtained. In a fresh and vigorous heart two minutes is usually the longest time that the rhythm may be entirely suppressed by stimulating the inhibitory nerves, even with a relatively strong interrupted current. In preparations showing a less vigorous rhythm, the inhibitory nerves seemed to be more effective. Thus in one case stimulation of the nerves produced complete arrest of the heart for twelve minutes.

The inhibitory nerves retain their function for a relatively long time after being severed from the brain. In excised hearts kept moist with plasma or sea-water I have found them functional for fifteen to twenty hours after removal of the heart from the body. This slow dying is also characteristic of the other tissues of this animal. With reference to the question whether the inhibitory nerves lose their function sooner than the augmentor-nerves, as Hering found to be the case in mammals, my experiments are not conclusive, owing to the difficulties encountered in working with the augmentor-nerves. The work on the inhibitory nerves comprised about ninety adults and twice that number of young specimens. In



FIGURE 7. — Record from first segment. Inhibition of the whole heart by stimulation of the inhibitory nerves in the pericardium after extirpation of the nerve-cord in the first two segments.

no single instance did the inhibitory nerves fail to produce the typical effects on the heart, as is sometimes the case in amphibians and mammals.

In the paper describing the local augmentor-reflex in the Limulus heart, it was stated that the heart may be inhibited by direct stimulation of the nerve-cord with a certain strength of the interrupted current, as well as by stimulating any of the main connectives between the nerve-cord and the lateral nerves. The view was expressed that the inhibition following the stimulation of the lateral connectives is due to escape of the current directly to the nerve-cord, rather than to stimulation of the afferent path of an inhibitory reflex mechanism, because in order to stimulate these connectives the electrodes are necessarily placed near the nerve-cord and the inhibition is, furthermore, not obtained except when very strong currents are employed. If the inhibition is due to the stimulation of the afferent path of a local reflex mechanism, we ought to obtain the same result on stimulation of the same nerve-fibres at a greater distance from the ganglion, but such is not the case. The inhibition produced by the strong interrupted current applied to the lateral connectives in the middle region of the heart may at times take the form shown in Fig. 8, a diminution in the strength of the beats without any change in the rate. By a further increase in the strength of the stimulus one usually succeeds in producing complete inhibition, or at least a

marked decrease in the rate of the beats. The form of inhibition shown in Fig. 8 can be accounted for by assuming that the weaker current has only a local effect, that is, suppressing the activity of the ganglion cells in the immediate vicinity of the electrodes only, while the stronger current affects the entire ganglion.

It was stated in the previous papers that the stimulation of the nerve-cord in the second, third, or fourth segments with a strength of the interrupted current sufficient to inhibit the heart behind the region stimulated produced tetanus of the heart anterior to the electrodes. The tetanus of the heart anterior to the region stimulated is

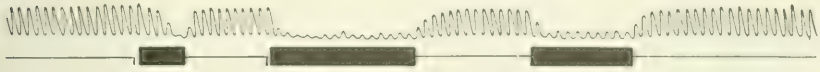


FIGURE 8. — Partial inhibition of the heart by stimulation of one of the connectives between the nerve-cord and the lateral nerve in the fifth segment. Strong interrupted current.

due to direct stimulation of the motor nerve-fibres that pass in the nerve-cord to the musculature of the anterior segments. To obviate this contraction, it is only necessary to isolate the nerve-cord in the first four segments. After such isolation the *stimulation of the cord at any point in the first four segments produces inhibition of the whole heart* and inhibition only. Now, in as much as the ganglion cells having the greatest automatism are situated in the middle region of the heart, and because of the further fact that the inhibitory nerves enter the nerve-cord in the second and third segments, it may be argued with good reason that the inhibition produced by stimulation of the nerve-cord in the first four segments is simply due to stimulation of the inhibitory nerves that are passing posteriorly in the nerve-cord to connect with the automatic ganglion-cells or to pass by means of the lateral connectives directly to the muscle. The stimulation of inhibitory nerves and nerve-endings in all probability also accounts for some of the inhibitory effects produced by stimulation of the lateral connectives or the nerve-cord itself in the region of greatest automatism; but the following facts point to the view that the interrupted current of a certain strength has a direct inhibitory or paralyzing effect on the automatic ganglion-cells themselves. In the first place some of the inhibitory effects produced by direct stimulation of the cord are not obtained by stimulation of the extrinsic inhibitory nerves. Attention has already been called to the dimin-

ished amplitude of the beats unattended by any change in the rate (Fig. 8). Furthermore, when the cord is being stimulated with a relatively strong interrupted current, the inhibition will the longer outlast the period of stimulation the stronger the stimulus. This was never observed on stimulation of the inhibitory nerves in the pericardium, or at a point near their junction with the brain. The prolonged inhibitory after-effects on stimulation of the cord can thus

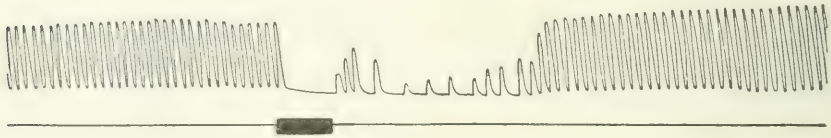


FIGURE 9. — Record from sixth segment. Prolonged inhibitory after-effects of stimulating the nerve-cord in the third segment with a moderately strong interrupted current.

not be ascribed to injury to the inhibitory nerves by the strong current, unless we assume that the nerves are more readily injured in the nerve-cord than on their course from the brain to the heart. Secondly, the strength of the current required to produce the inhibition is so considerable that it is highly probable that the automatic ganglion-cells are affected by it. The inhibition is obtained after the inhibitory nerves have been thrown out of action by atropin (Fig. 10), which is an additional evidence that it cannot be due solely to the stimulation of the inhibitory nerve-fibres that pass from the brain to the nerve-cord.

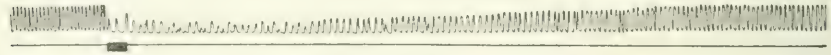


FIGURE 10. — Record from heart on direct stimulation of the nerve-cord in the third segment after the inhibitory nerves had been paralyzed by atropin. Moderately strong interrupted current.

The inhibition produced by the strong induced shock and the strong interrupted current in the heart of the hagfish (Bdellostoma)[1] was interpreted by me as due to the direct action of the electrical current on the muscle, on the assumption that the heart-beat is myogenic. On the myogenic theory, one can scarcely interpret this inhibition in the hagfish heart in any other way, because of the absence of cardio-regulative nerves in this animal, and because of the great strength of the induced current required to produce the inhibition. Inhibitory phenomena, exactly similar to those in the hagfish heart,

[1] CARLSON: Zeitschrift für allgemeine Physiologie, 1904, iv, p. 259.

are also produced by the strong induced current in the heart of some molluscs, which, according to all evidence, is not provided with inhibitory nerves. My results on the Limulus heart have led me to believe that the cause and co-ordination of the heart-beat, both in invertebrates and vertebrates, are to be sought in the nervous tissue of the heart. Now, inasmuch as ganglion cells, and probably also nerve-fibres, are more readily affected by the induced current than is muscle, the strong current required to produce the inhibition in the hagfish heart, and in the heart of some of the lower gasteropods, must therefore act on the nerve-cells. The slight "tonus" contraction that sometimes accompanies this inhibition may be due to the direct action of the current on the muscle. The inhibitory action of the strong induced current on the automatic ganglion cells probably partakes more of the nature of a paralysis from over-stimulation, than of the true inhibition. This question will be considered more fully in connection with my results on the molluscan heart.

4. The Inhibitory Nerves Act on the Automatic Ganglion and not on the Heart Muscle.

The action of the vagi on the vertebrate heart, as determined by the earlier observations of Weber, and particularly by the more recent work of Gaskell and Engelmann and their students, consists in diminution of the rate and power of contraction and conduction, and in diminution of the excitability of the heart to direct stimulation. It is plain, from the previous section, that so far as regards the influence on the rate and the power of contraction, the physiology of the cardio-inhibitory nerves in Limulus does not differ from that in the vertebrates. It is also easy to prove that the inhibitory nerves in Limulus produce a diminished excitability of the heart to direct stimulation, as well as diminished power of conduction. Diminished power of conduction in the heart during the stimulation of the inhibitory nerves is shown by the fact that the contraction produced by direct stimulation of the heart at any point usually does not spread over the whole heart, but is confined to the region stimulated. It is, however, possible to obtain similar local contractions in response to direct stimulation, even in hearts that are beating with the normal rhythm and are not under the influence of the inhibitory nerves. Thus, by gently touching the heart at any point in the first two or the last two segments, the extra-contraction produced may be confined to the stimu-

lated region. More often, however, the contraction spreads over the whole heart, and this is always the case when the stimulus is of considerable strength. A strength of the stimulus that invariably produces a contraction involving the entire heart, produces only a local contraction in the inhibited heart. A strong stimulus applied to the inhibited heart appears in some way to hasten the "escape" of the heart from the inhibitory influence. Thus, when the heart is brought to a complete standstill by stimulation of the inhibitory nerves, a weak induced shock, sent through the heart from side to side in the fifth or sixth segments, causes a weak contraction of these segments, or at the most, these segments, together with the adjacent anterior and posterior ones, and after this weak and local beat the heart continues in complete inhibition as before; but when a very strong induced shock is thus applied, the contraction usually spreads over the whole heart and is followed by a rhythmical series of beats, even though the stimulation of the inhibitory nerves is continued. These local contractions in the Limulus heart, in response to direct stimulation during inhibition, are to be compared to the local contractions in the inhibited auricles of vertebrates, rather than to the diminution or abolition of conduction between the auricles and the ventricles.

The variation in the amplitude of the response to direct stimulation during inhibition is taken as an index of the influence of the inhibitory nerves on the excitability of the heart. In incomplete inhibition, the inhibitory nerves appear to diminish the power of contraction itself. That may be accomplished in either one of these two ways, viz., the nervous impulses reaching the heart-muscle are diminished in strength, or the heart-muscle is so altered that the nervous impulses produce submaximal contractions. Such a change in the heart muscle could not be distinguished from a decreased excitability, and if this second alternative is the mechanism by which the inhibitory nerves produce a diminution in the amplitude of the beats in incomplete inhibition, the diminutive beats are themselves sufficient evidence of the diminution of the excitability of the heart during inhibition. But if the inhibitory nerves produce the diminutive beats of incomplete inhibition by diminishing the strength of the nervous impulses from the ganglion-cells, the excitability of the heart-muscle must remain unaffected, and the changes in the response of the heart to direct stimulation must be referred to changes in the excitability of the nervous mechanism. It is plain, however, that in such experi-

ments we cannot separate the effects of the inhibitory nerves on excitability, from that on the capability of response of the nervous mechanism, nor can we separate these from that on the power of conduction, unless the power of conduction could be altered as regards the rate of propagation of the nervous impulse without any change in the intensity or stimulating power of the impulse conducted. If the so-called automatic activity of the ganglion-cells in the heart is in reality a series of reflexes, the diminution of the beats by the inhibitory nerves might itself be construed as a diminution in the excitability of the ganglion-cells; but if the ganglion is automatic, in the strict sense of that word, it is conceivable that their excitability to direct stimulation might be altered without any alteration in the power of nervous discharge. If the inhibitory nerves diminish the

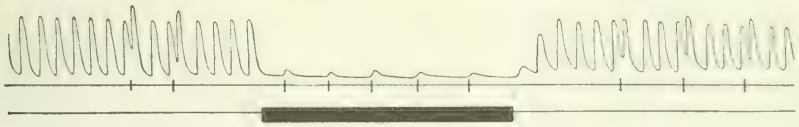


FIGURE 11. — Record showing change in the excitability and contractility of the heart produced by stimulation of the inhibitory nerves. The lower signal shows duration of stimulation of the inhibitory nerves, upper signal direct stimulation of the heart with single induced shocks.

power of nervous discharge, we have no means of knowing whether or not the excitability of the cells is at the same time reduced; and the power of conduction being diminished, we have no means of knowing whether the diminution in excitability and power of nervous discharge must be referred to actual change in these properties, rather than to this change in conduction, because the latter would necessarily produce the two former effects. But for the present purpose, we may speak of the changes in the response of the heart to direct stimulation during inhibition, as alterations in excitability.

A mechanical stimulation, or an induced shock of moderate strength, produces a stronger contraction when sent through the heart during any phase of the automatic beat, than when sent through the inhibited heart. A strength of the stimulus just sufficient to affect the heart when not under the influence of the inhibitory nerves, is without visible effect when applied to the heart in complete inhibition. Inhibition diminishes, but does not abolish, excitability. So far, then, there is a complete identity between the Limulus and the vertebrate heart. Typical tracings, showing this diminution in excitability, are reproduced in Figs. 11 and 12. In

Fig. 11, the same strength of the induced shock produces beats of thrice the amplitude produced during inhibition. In Fig. 12, relatively weak break-shocks are employed. Yet when the shocks are sent through the heart at the beginning of systole, supermaximal beats are produced, while the shocks are ineffective during the stimulation of the inhibitory nerves.

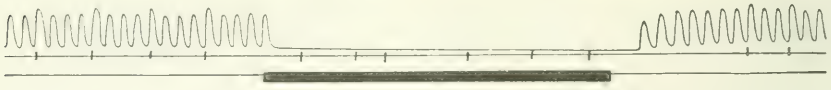


FIGURE 12. — Tracing showing greatly diminished excitability of the heart during stimulation of the inhibitory nerves. Upper signal shows point of direct stimulation of the heart with weak induced shocks.

The inhibitory effects on the rhythm of stimulating the inhibitory nerves appear to be maximal at the very beginning of stimulation. There never appears to be any gradual overcoming of the rhythm. Yet tracings like the one reproduced in Fig. 13 show that the greatest diminution of excitability and contractility is not reached at once, or simultaneously with the cessation of the rhythm. In Fig. 13, the lower signal shows the duration of the stimulation of the inhibitory nerves with a strength of the interrupted current just sufficient to produce complete inhibition. The upper signal gives the points of stimulation of the heart with break induced shocks of uniform intensity. The gradual diminution in excitability is shown both at *A* and *B*.

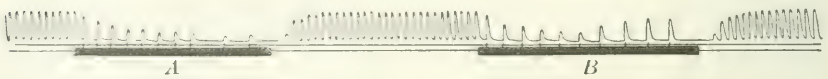


FIGURE 13. — Tracing from the fifth segment, showing variations in excitability of the heart produced by the inhibitory nerves. Upper signal, stimulation of the heart with single induced shocks; lower signal, stimulation of the inhibitory nerves with a weak interrupted current.

The gradual return of excitability at *B* is very similar to the gradual "escape" of the heart from the inhibition, or the gradual return of the rhythm on cessation of the stimulation of the inhibitory nerves. The stronger the stimulus applied to the inhibitory nerves, the quicker this state of lowest excitability is reached. To obtain records like those in Fig. 13, the interrupted current must not be stronger than just sufficient to produce complete inhibition.

So far, then, the inhibitory nerves produce the same effects on the heart of Limulus as do the vagi on the heart of the higher verte-

brates. The evidence that this action is on the ganglion and not on the heart-muscle may be briefly stated as follows:

(1) The inhibitory nerves enter, not the heart-muscle, but the nerve-cord on the dorsal side of the heart, and we have seen that this is the automatic centre of the heart-rhythm. The connection with the ganglion is made in the region of the second and third pairs of ostia. The inhibitory nerves, entering the nerve-cord of the anterior end of the heart, must, in order to reach the heart-muscle in that region, pass posteriorly in the nerve-cord to the fifth or sixth segments, and there connect with inhibitory ganglion-cells which, in turn, send their processes to the lateral nerves, and thus ultimately to the muscle, or the nerves must take this circuitous route without this "relay"; because the entire nerve-cord may be extirpated anterior to the point of entrance of the inhibitory nerves, and the remainder of the cord, dissected free from the heart down to the fifth segment, thus making it impossible for inhibitory fibres to reach the heart-muscle of the first two segments except along the lateral nerves, yet after this lesion the stimulation of the inhibitory nerves inhibits the heart-rhythm not only of the middle and posterior portion of the heart but of the anterior segments as well. After such a lesion of the nerve-cord, the strength of the contractions in the anterior segments is very much diminished; but these segments continue to beat in synchrony with the rest of the heart as long as the lateral nerves are intact. It would not seem probable that the inhibitory nerves which enter the ganglion in the anterior region of the heart should take such a roundabout path to reach the muscle.

(2) *The response of the heart to direct stimulation when quiescent in prolonged diastole, due to strong stimulation of the inhibitory nerves, is similar to the response of the heart to the same stimulus after extirpation of the nerve-cord.* The diminution of excitability to direct stimulation is almost identical in the two cases. *Complete inhibition thus simply means the throwing out of function of the nerve-cord.* It was stated in my former paper that extirpation of the nerve-cord diminishes the excitability of the heart as compared with that with the nerve-cord intact. This is invariably the case, that is, provided the nerve-cord is alive and functional. That fact led me to compare in the following manner the excitability of the heart during inhibition with that after extirpation of the cord. The heart with the nerve-cord intact is prepared for accurate graphic registration, and the response of the heart to induction shocks of a given intensity, sent through

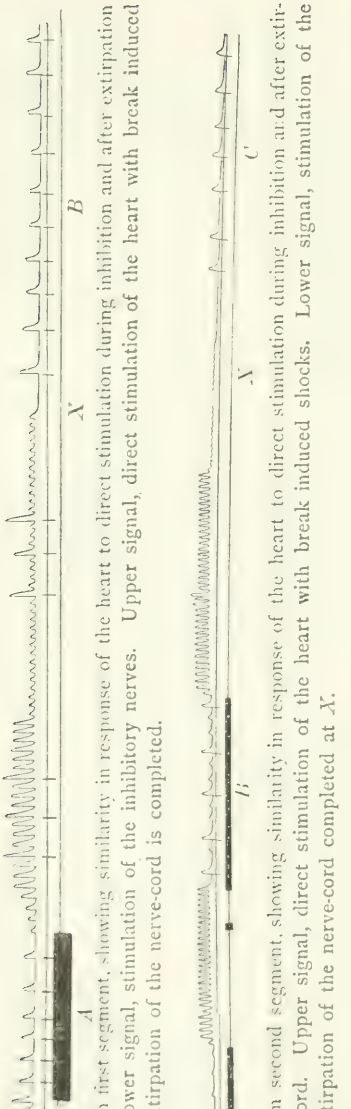


FIGURE 14. — Tracing from first segment, showing similarity in response of the heart to direct stimulation during inhibition and after extirpation of the nerve-cord. Lower signal, stimulation of the inhibitory nerves. Upper signal, direct stimulation of the heart with break induced shocks. At *X*, the extirpation of the nerve-cord is completed.

FIGURE 15. — Tracing from second segment, showing similarity in response of the heart to direct stimulation during inhibition and after extirpation of the nerve-cord. Upper signal, direct stimulation of the heart with break induced shocks. Lower signal, stimulation of the inhibitory nerves. Extirpation of the nerve-cord completed at *X*.

the heart during diastole, recorded. The inhibitory nerves are now stimulated, and during this stimulation induction shocks of the same intensity are sent through the heart at the same point. The nerve-cord is now extirpated, an operation which is followed by an immediate and permanent diastole, and the heart again stimulated with the same strength of induction shocks. As already stated, the amplitude of the contractions produced by the direct stimulation during the inhibition is practically the same as that produced by the same stimuli after extirpation of the nerve-cord. This, it appears to me, is a crucial experiment. I have repeated it on more than fifty hearts, obtaining the same results in every one, without exception. In some preparations the amplitude of the contractions during the inhibition was a little less, in the majority of the preparations a little more than after the removal of the ganglion, but the variations were in every case so slight as to leave no doubt that the inhibition of the heart is practically equivalent to rendering the ganglion incapable of functioning. The second conclusion to be drawn from this experiment is this, that the ganglion-cells are much more readily excited than the nerve-fibres or the heart-muscle itself. The nervous tissue, particularly the ganglion-cells, is therefore the first involved in the peculiarities of the response of the heart to direct stimulation.

Two typical records obtained by the above method are reproduced in Figs. 14 and 15. In both these tracings the lower signal shows the stimulation of

the inhibitory nerves with the interrupted current, the upper signal the direct stimulation of the heart with single-induced shocks. In Fig. 14 the induced shocks sent through the heart at the beginning of systole, when the heart is not under the influence of the inhibitory nerves, produce supermaximal beats. At *A* the rhythm is completely inhibited, and the same stimuli produce only diminutive beats. At *X* the extirpation of the nerve-cord is completed. The contractions caused by the same induced shocks at *B* vary somewhat in amplitude, but on the whole they are of the same strength as those at *A*. The same comparations hold good for the contractions at *B* and *C* in Fig. 15.

This diminution of the excitability of the heart after extirpation of the ganglion is permanent, and can therefore not be ascribed to temporary stimulation of inhibitory nerve-fibres that might pass from the ganglion to the heart-muscle. Nor are there, so far as I can see, any sources of error in the conditions of the experiment which might account for the striking similarity in the heart's excitability when in a state of complete inhibition and with the ganglion removed, and thus obviate the above conclusions.

(3) Atropin, nicotin, and curare paralyze the inhibitory nervous mechanism in the vertebrate heart. Nicotin and curare are not supposed to paralyze the inhibitory nerve-endings in the muscle, because it is claimed that the heart may be inhibited on direct stimulation after the stimulation of the vagi has been rendered ineffective by these drugs. Atropin accelerates the heart, and this acceleration is usually ascribed to the paralysis of the inhibitory nerve-endings in the muscle. This action of the alkaloid would be analogous to its action on the nervous mechanism of the sphincter iridi, and appears to be further supported by the claim that after paralysis of the vagi by atropin the heart cannot be inhibited by direct stimulation.

The action of drugs on the heart of Limulus will be considered in a subsequent paper, but in this connection it may be stated that atropin, nicotin, and curare paralyze the inhibitory mechanism of the Limulus heart just as in the heart of vertebrates. This paralyzing action of the alkaloids, always preceded by and accompanied by augmentation of the rhythm, is perhaps less quick and certain than in the case of the heart of the snake or the tortoise, which animals I used for comparations. There does not appear to be any decided difference between these alkaloids in their power to paralyze the inhibitory mechanism in the Limulus heart. The function of the inhibitory

nerves return rather quickly on bathing the heart in plasma or sea-water after being subjected to the action of the drugs. A tracing showing the stimulating action of nicotin on the rhythm and the accompanying paralyzing action on the inhibitory nervous mechanism is reproduced in Fig. 16. At *X* a solution of one part of the alkaloid to two thousand parts of plasma is applied to the nerve-cord. The stimulating action is in this particular case unusually great. The signal shows the point of stimulation of the inhibitory nerves. At *A* the inhibitory nerves are able to greatly counteract the stimulating action of the drug, although not able to bring the heart to complete standstill.

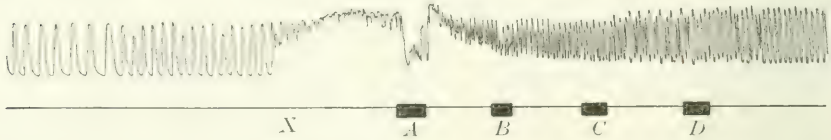


FIGURE 16. — Tracing from first segment. *X*, application of a weak solution of nicotin to the nerve-cord. *A*, *B*, *C*, *D*, stimulation of the inhibitory nerves, showing paralyzing effects of the alkaloid on the inhibitory mechanism when applied to the nerve-cord.

At *B* the function of the nerves is almost, and at *C* and *D* it is completely lost. Records like these also go to show the paralysis of the inhibitory nerves is not simply due to the stimulation of the automatic ganglion by the drug to such an extent that the influence of the inhibitory impulses is completely obscured, for at *A* the ganglion is being more strongly stimulated than at *D*, yet the inhibitory nerves are effective in the former case but not in the latter. They also show that the augmentation is not due to the paralysis of the inhibitory nervous mechanism, an explanation usually favored in the case of the acceleration of the vertebrate heart by atropin.

These alkaloids paralyze the inhibitory mechanism only in case they come in contact with the ganglion on the dorsal side of the heart; when they are allowed to act on the muscle and the nerves and nerve-endings, only the inhibitory nerves are not thrown out of function. The reader is referred to my paper in Science[1] for a diagram and description of the preparation of the Limulus heart for the study of the effects of solutions on the ganglion-cells apart from that on the muscle and the nerve-endings. This separation can be made absolute in the Limulus heart. After the nerve-cord has been extirpated from the first three segments, and a portion of the heart-muscle removed in

[1] CARLSON: Science, 1904, xx, p. 684.

the third segment, leaving the two anterior segments in connection with the rest of the heart by means of the lateral nerves only, the first two segments of the heart may be bathed in 0.5 per cent atropin for hours without any effect on the inhibitory mechanism. Now, this is the condition. If the inhibitory nerves acted, not on the ganglion-cells in a way to diminish or abolish their activity, but on the heart-muscle in a way to render it less able to respond to impulses from the ganglion-cells, the paralysis of the inhibitory nerve-endings in the first two segments by atropin would have this result, that this portion of the heart would continue its rhythm unaffected, while the posterior portion of the heart, not acted on by the drug, would be inhibited by the stimulation of the inhibitory nerves. But this never happens. The part of the heart subjected to the action of the alkaloid keeps in perfect synchrony with the rest of the heart; on slowing or cessation of the rhythm of the hind portion of the heart, a similar change takes place in the rhythm of the anterior portion. Atropin, nicotin, and curare, in strengths that do not kill the muscle and the nerve, do not paralyze the inhibitory mechanism in the heart of Limulus unless they come in contact with the ganglion. Their action is therefore of a nature to prevent the inhibitory impulses from reaching the automatic ganglion-cells or to diminish the excitability of the automatic cells to the inhibitory impulses.

(4) If inhibitory nerves pass from the nerve-cord to end in the muscle, it ought to be possible to produce inhibition by stimulation of these fibres on their course from the nerve-cord to the muscle, or by stimulation of their endings in the muscle, but this is not the case, or at least my numerous attempts in that direction failed. Inhibition may be produced by stimulation of the ganglion directly; but stimulation of the nerves that pass from the ganglion to the muscle, or stimulation of the heart in any segment from which the nerve-cord has been removed, produces contraction and contraction only. Taking all these facts together, there can be no doubt that cardiac inhibition in Limulus falls within the category of inhibition of automatic or reflex neural processes in the central nervous system. Weber's theory of cardiac inhibition is demonstrated for Limulus at least. It remains to be shown that the change in the electrical tension in the heart observed by Gaskell on stimulation of the vagi is due to changes in the nervous tissue and not to changes in the muscle. This work was begun at Wood's Hole last season, but could not be carried to completion owing to technical difficulties.

Now that it has been shown that effects of the vagi on the vertebrate heart are duplicated in the heart of Limulus on stimulation of the inhibitory nerves, and that the action of the inhibitory nerves is on the ganglion in the heart, and not on the heart-muscle, it seems to me that the burden of proof falls on those who would maintain that the mechanism of inhibition of the vertebrate heart differs from that of the Limulus heart, in as much as all the facts can be accounted for by the nervous nature of the inhibition. There appears to be no sufficient reason for holding that the nervous mechanism of other parts of the vascular tube, particularly the arterioles, is any different from that of the heart. The vasodilator fibres are in all probability homologous with the cardio-inhibitory fibres and their mechanism of action the same. But while it is very probable that cardiac inhibition and vasodilatation are to be referred to the physiological processes known to take place in nerve-centres, namely, inhibition of one neural process by another, it should not be forgotten that the well-known observations of Biedermann on the nerve to the chelæ of the crayfish appear to establish a case where inhibitory nerve-fibres end and act directly on the muscle. According to Biedermann a motor and an inhibitory nerve-fibre end on each individual muscle-cell. There appear to be no ganglion-cells on the course of the nerve or in the muscle which would allow for a nervous mechanism of inhibition. Such is, however, the case in the corresponding nerve-muscle mechanism in Limulus. In the last joint on the course of the nerves to the ambulatory appendages of Limulus are found numerous ganglion cells. These cells even function as a peripheral reflex centre for closing the claw.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS.[1] I.—GENERAL METHODS.

By TORALD SOLLMANN.

(With the Collaboration of R. A. HATCHER.)

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A. Introductory.

A KNOWLEDGE of the mechanical phenomena occurring in the kidney would seem to be a necessary prerequisite to the discussion of any theory of urine secretion. These mechanical actions cannot be readily controlled in intact animals, and the problems can be best approached by perfusing excised kidneys in which experimental conditions can be modified at will. This has been done by numerous investigators, and some important conclusions have been reached. The experiments have generally been performed with defibrinated blood. This has several technical inconveniences; the ureter-flow, for instance, is always very small. Perfusion with blood also permits the continuance of a considerable amount of vital activity, as we shall show later. The problems of the mechanical actions are greatly simplified by excluding the vital phenomena as completely as possible. This we have tried to do by various means, mainly by using plain saline solutions for perfusion.

Historical notes on kidney perfusion.—The perfusion of the excised kidney with defibrinated blood seems to have been first attempted by

[1] Preliminary communications of some of the experiments treated in these papers were made in this journal, 1903, ix, p. 460, and at the meeting of the American Physiological Society, December, 1903. (This journal, 1904, x, p. xxv.)

Loebell[1] and repeated by Bidder,[1] and later by Munk and Senator[2] and other investigators.[3] Jacobj[4] perfected the technic by using intermittent pressure for the perfusion. This has also been employed by Brodie.[5] When we began our experiments we knew of no work on the perfusion of kidneys with saline solutions. Since then, however, we have found that it was used by Ludwig[6] as early as 1863, to explain the effect of vein compression. Other experimenters have also used the method incidentally, but no one appears to have made any further experiments bearing on the present subject.

We have found the method admirably adapted to the study of our problem. Its simplicity leaves nothing to be desired, and the kidney can be exposed to the greatest variety of experimental conditions during many hours. A flow of liquid from the renal vein and from the ureter sets in at once on starting the perfusion, the strength of the flow varying with the experimental conditions.

The vein and ureter flow is very different for the kidneys of different animals, even under identical conditions. The differences of the vein flow are doubtless referable to differences in the anatomical arrangement of the blood vessels; for instance, the flow is usually much more free in large kidneys. These variations in the circulation entail corresponding variations in the ureter flow. There can be no doubt, however, that the filtration's permeability is also variable. In Experiment 64, for instance, there was a small ureter flow with a good vein flow. The ureter flow also lessens with the time elapsing after the removal of the kidneys from the body, independent of circulatory changes. However, the two kidneys of the same animals usually behave nearly alike.

B. Evidence that the Ureter Fluid is a Filtrate and is not Produced by the Rupture of Blood Vessels into the Tubules.

We must, of course, be certain of this point, before we can accept our experiments as evidence of the effect of our conditions on urine filtration. The proof is quite definite.

[1] Loebell (1849) and Bidder, E. (1862): Cited in Henle and Meissner's Berichte, 1863, p. 324.

[2] Munk, I.: Archiv für pathologie Anatomie, 1877, cvii, p. 291; cxi, p. 434; Munk and Senator: *Ibid.*, 1888, cxiv, p. 1.

[3] See Pfaff, F., and Vejux Tyrode, M.: Archiv für experimentelle Pathologie und Pharmakologie, 1903, xlix, p. 324.

[4] Jacobj and v. Sobieranski: *Ibid.*, 1892, xxix, p. 25.

[5] Brodie, T. G.: Journal of physiology, 1903, xxix, p. 226.

[6] Ludwig: Cited from Henle and Meissner's Berichte, 1863, pp. 323 to 328.

(a) The perfusion pressure never exceeds the normal blood pressure, so that rupture is, *a priori*, improbable.

(b) If blood mixtures are circulated after the saline solution, the ureter fluid remains free from corpuscles; and this even if the saline perfusion has been continued five hours, or if the kidneys have lain several days on ice.

(c) If the composition of the perfusing fluid is altered, changes in the composition of the ureter fluid are only completed after some twenty to forty minutes.

(d) If the kidneys are used one or more days after excision, the ureter flow is very small, whereas the vein flow may be even better than on the first day. The lessened ureter flow could not be explained if a rupture existed; whereas it is easy to understand how the rapidity of filtration would be influenced by the post-mortem coagulation of the protoplasm.

C. Persistence of the Vitality of the Kidneys.

This is a question of importance when it is desired to reduce the kidneys to a purely mechanical basis. The sensitiveness of the kidney to all kinds of injuries, and its peculiar greed for oxygen, would argue that vital actions must be at least greatly diminished during perfusion with saline solutions. Pfaff and Vejux Tyrode[1] have indeed shown that the mere defibrination of the blood causes the kidney to act abnormally, even in the body. However, the injury is not profound, since the functions are recovered on resuming the circulation of normal blood. There is, indeed, absolute proof that a certain degree of vitality is maintained in the excised kidney for many hours. This evidence can be classified as follows:

1. Differences in composition of the perfusing and ureter fluids. — (a) *Chlorides.* — Munk[2] has shown that there is a small, but distinct increase in the percentage of chlorides in the ureter fluid over that of the serum of the perfusing blood. When the latter is mixed with sodium sulphate, I have found that the ureter fluid contains less chlorides than the serum. When, however, the kidney was perfused with mixtures of isotonic sodium chloride and sulphate solution (without blood), the chlorine of the perfusing and ureter fluid were exactly alike.

Whilst these experiments seem to show that the kidneys perfused with blood mixtures retain to a certain degree the power of regulating the chloride excretion, this power is certainly greatly reduced by excision of

[1] Pfaff, F., and Vejuz Tyrode, M.: *Loc. cit.*

[2] Munk: *Loc. cit.*

the kidneys. The greatest retention which I have observed occurred in Experiment 37, in a kidney perfused with beef's blood diluted with two parts of sodium sulphate solution. The serum of the mixture contained 0.402 per cent of sodium chloride; the ureter fluid, 0.265 per cent. In the intact animal, the injection of sodium sulphate, in amounts which dilute the blood much less than the above, gives urines in which the percentage of sodium chloride is generally less than 0.02 per cent, even when the blood of the animal is defibrinated by Pick's method. This shows that the inefficient chloride retention of the excised kidney cannot be referred to the defibrination of the blood.

(b) *Urea.* — Jacobj[1] found the urea of the ureter fluid 0.4 per cent, whilst that of the perfusing blood was 0.05 per cent.

(c) *Albumen.* — The ureter fluid is always albuminous when blood is used, but the percentage of proteid is considerably less than in the blood (0.82 per cent, Jacobj). I have found it even very much smaller. In Experiment 137, the ureter fluid was collected during the perfusion of the kidney with defibrinated dog's blood, diluted with two parts of 1 per cent sodium chloride solution, from two to three hours after the death of the animal. The serum of the blood mixture contained about 1.095 per cent of proteid; the proteid of the ureter fluid amounted only to 0.007 per cent, *i. e.*, 1 ÷ 156 of the percentage of the serum. The ureter fluid was almost colorless. Its tint corresponded to that of a 1 : 10000 dilution of the blood.

The elimination of a practically proteid-free fluid from the ureter can scarcely be used as an argument for the vitality of the kidney, since Martin[2] has shown that gelatin filters are also able to yield a proteid-free fluid.

(d) *Reaction.* — When blood is perfused through the excised kidney, the first drops of the ureter fluid have an acid reaction, although this soon becomes alkaline.

2. Hippuric acid synthesis. — Schmiedeberg and Bunge[3] determined that this takes place even forty-eight hours after death, if the kidneys are perfused with blood; it does not occur, however, even in fresh kidneys, when perfused with serum or with 2 per cent sodium chloride; nor does it occur in bruised kidney tissue, even in the presence of blood. These experiments show that the kidney preserves the vital functions concerned in this synthesis for at least forty-eight hours, but that they cannot be exercised in the absence of oxygen.

3. The excretion of pigment. — The experiments of Heidenhain have shown that when sulphoindigate of sodium is injected into living

[1] Jacobj: *Loc. cit.*

[2] Martin, C. J.: Journal of physiology, 1896, xx, p. 364.

[3] Schmiedeberg and Bunge: Archiv für experimentelle Pathologie und Pharmacologie, 1876, vi, p. 233.

animals, the pigment is localized in the kidneys in the cells of the convoluted tubules and in those of the broad segments of Henle's loop. Chrzonszczewski and Wittich, on the other hand, showed that carminate of ammonium or sodium is localized in the living kidney exclusively in the glomerular tufts. In dead kidneys, all cells are stained diffusely by either pigment.

This differential reaction may, therefore, be utilized to determine the vitality of the kidneys. Blumberg (1889, quoted by Jacobj) tried the experiment on excised kidneys perfused with blood, and found that death occurred rapidly. Jacobj and v. Sobieranski,[1] in a more extensive investigation, found that both the glomerular and the tubular epithelium preserved their vitality even after four hours, when the perfusion of the kidney was made with intermittent pressure. With constant pressure, they found that some portions remained alive, whilst others had died. The latter result is attributed to obstruction of some of the vessels by gravitated blood, etc.

4. Reduction of hæmoglobin. — Even the earliest observers (Loebell, Bidder, etc.) emphasize the markedly venous condition of the blood which has passed through the kidney. There is perhaps no organ in which this can be so well demonstrated. The degree of reduction is, of course, inversely proportional to the rapidity of the circulation.

I have had numerous occasions to observe how this reducing power is affected by various conditions. I have not made any oxygen estimations, but have merely watched for the change of color, the arterial and venous cannulas lying side by side. This method is not at all delicate, but is sufficiently sensitive for the present purposes.

From these observations, I would say that the reducing power is distinctly less after perfusing with 1 per cent sodium chloride for two hours, but a considerable amount of reduction occurs even after six hours. The time at which it disappears seems to vary with different kidneys. In Experiment 142, no reduction occurred eighteen hours after death; in Experiments 139 and 140 appreciable reduction was present twenty-two and twenty-nine hours after death. In all cases, however, the reducing power appears to be entirely lost when the kidneys have been kept on ice for two days. A considerable amount of reduction occurs after the kidneys have been perfused for about an hour with 2 per cent sodium chloride, or for three-quarters of an hour with sodium fluoride (0.3 per cent in 1 per cent sodium chloride).

The above data refer mainly to the vitality of the epithelium. They show that some degree of life persists under very unfavorable conditions; but they also show that the vitality is severely injured

[1] JACOBJ and v. SOBIERANSKI: *Loc. cit.*

even when the greatest precautions are observed. In view of the latter fact, there can be but little doubt that the fluid flowing from the ureter, on perfusion with saline solution, is a physical filtrate, not a vital secretion. This is also confirmed by the behavior of the ureter pressure (which will be considered in the second paper). It is completely established by the fact that the ureter flow becomes even more copious when 80 per cent alcohol or 5 per cent sodium chloride is perfused, which would, of course, lessen or abolish the vitality. It is true, on the other hand, that the ureter fluid is greatly lessened by the death of the kidney, *e. g.*, by letting the kidney lie over night, or by perfusing mercuric chloride or picric acid. But it seems to me that this is not due to the absence of vital action, but to post-mortem coagulation.

Passing now to the persistence of *the vitality of the vessels*, I have found the following:

1. **Dilator reaction of blood.** — In an investigation which will be described in a later paper, I have observed that viscidity of the perfusing solution greatly diminishes the flow from the renal vein; with egg albumen, this occurs whether the kidney has been recently excised, or whether it has lain for several days. Using one part of defibrinated blood to three parts of 1 per cent sodium chloride solution, the flow from the vein is almost stopped if the kidneys have lain for two days; *if, however, the fresh kidney is used, the vein is generally increased*, often to two or three times its previous height. This increase of vein flow in the face of the increased viscidity argues for a dilation. The dilator reaction is also obtained after six hours perfusion with 1 per cent sodium chloride (although there is reason to believe that it diminishes with time). The time at which the dilator reaction disappears seems to coincide with the disappearance of the hæmoglobin reduction (see above).

 The dilator reaction persists (although it is perhaps diminished) after an hour's perfusion with 2 per cent sodium chloride or with sodium fluoride (0.3 per cent).

2. **Adrenalin reaction.** — A very marked slowing of the vein and ureter flow and a great drop in the oncometer occurs when adrenalin (1 : 50000) is added to the perfusing blood or salt solution. The reaction appears to diminish with the time elapsing after death, but in some kidneys it is still quite marked after several hours' perfusion with 1 per cent sodium chloride solution. Eventually, however, the constrictor action disappears, sometimes very early. At times it is replaced by a dilator reaction. The constrictor reaction is also destroyed, or at least greatly diminished by sodium fluoride and by 2 per cent sodium chloride.

3. Hydrocyanic acid reaction. — When this poison is added, in the proportion of $\frac{1}{2500}$, to either blood or 1 per cent saline solution, it causes a dilation of the renal vessels, shown by a very conspicuous increase of the vein and ureter flow, and of the oncometer. This reaction is not abolished by 2 per cent sodium chloride solution, nor by sodium fluoride. It is greatly diminished or abolished two days after excision.

These data indicate that the renal vessels also preserve their vitality under very adverse conditions; but they show at the same time that the vitality is considerably less after the perfusion of saline solution. The vitality of the vessels seems to be completely abolished within two days after excision. Such kidneys may, therefore, be used to control whether an observed effect on the vessels need to be referred to vitality. They are, however, unsuitable for investigation of the filtration phenomena. The latter may be controlled by the perfusion of 2 per cent sodium chloride, which at least severely injures any vital phenomena on the part of the epithelium.

Wherever possible, we have also tried to imitate the observed phenomena on artificial models, to show that a mechanical explanation is sufficient.

D. Methods of Investigation.

Preparation of kidneys. — All the experiments are made on dogs, which are anæsthetized by a large dose of morphine and a just sufficient amount of ether. In many experiments the blood was first withdrawn and defibrinated. The kidneys are then freely exposed by a long median incision and a transverse incision below the border of the ribs. Each kidney is then treated separately. The fatty envelope is stripped off leaving the fibrous capsule intact. The ureter and the renal vessels are then isolated and cleaned. A cannula is inserted into the ureter; the free end of this cannula is connected with a bent delivery tube, drawn to a point of about 1 mm. diameter. The tube of the perfusion apparatus, filled with saline solution (generally 1 per cent sodium chloride), and under the pressure of 100 cm. to 140 cm. of water, is then laid handy. The renal artery is now tied close to the aorta, and a glass cannula fixed in its peripheral end. This cannula is filled with saline solution, connected with the perfusion tube, and flushed with the saline solution until the fluid in the vein appears almost colorless. This is for the purpose of preventing intravascular clotting (if this is omitted a longer time is needed to establish a constant flow; but the final result is the same (Experiments 78 and 79)). The perfusion is then stopped and the renal vein is tied close to the vena cava, and another glass cannula inserted into it, toward the kidney. The vessels are then cut beyond the cannulas, and the kid-

ney is lifted out and transferred to a small bench. A short, bent glass tube, tapering to a delivery orifice of about 3 mm. diameter, is attached to the vein cannula for the collection of the outflowing fluid. The arterial cannula is firmly fixed by a clamp, care being used that none of the vessels are twisted. The kidney is protected from drying by a skin muscle flap, taken from the abdominal wall, according to the suggestion of Munk.[1] This is omitted when the oncometer is employed. The perfusions were carried on at the room-temperature, which was practically constant during each experiment.

The fluid flowing from the vein and ureter is collected in beakers, the rapidity of flow being estimated by counting the rate of dropping, or by measuring the time required to collect a convenient quantity of fluid, generally 15 c.c. It may be remarked that the drops from the ureter are only one-third to one-half the size of those from the vein, on account of the smaller orifice of the delivery tube.

Accidents. — There is always considerable leakage from the surface of the kidneys through the collateral vessels. This does not influence the results.

It sometimes happens that the renal artery divides so close to the aorta that it is impossible to introduce a cannula in the common trunk. In these rare cases the cannula was placed in the larger branch, the other having been tied. This also does not affect the results.

Care must be used to prevent the entrance of air into the renal vessels, for this causes sudden diminution of the vein and ureter flow, and renders the kidney useless for about an hour. At the end of this time, the flow has usually returned to the original, the vein recovering more promptly than the ureter flow (for instance, in Experiment 62).

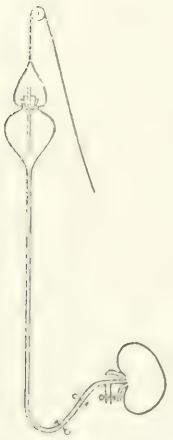


FIGURE 1. — Perfusion arrangement.

Perfusion apparatus. — The arrangement of the apparatus is shown in Fig. 1. The bulbs have a capacity of 100 c.c. to 2 l., and are suspended from pulley attached to a crossbeam, at a height of 1.5 m. above the kidneys. (The injection pressure is measured between the kidneys and the lower end of the tube in the reservoir. This glass tube has the further advantage of keeping the fluid aerated.) The connection between the bulb and the kidney is made by alternate pieces of rubber and glass tubing, so that air bubbles can be readily detected. The T-piece allows the removal of air bubbles. It is also used when different solutions are to be alternated.

Oncometer. — We employed a somewhat simplified modification of the ordinary oncometer which was sufficiently delicate to indicate differences

[1] MUNK: *Loc. cit.*

of 5 cm. in the level of the reservoir, even when the vein was open. The pressure in the oncometer was usually kept between 15 cm. and 30 cm. of water.

Other modifications of the apparatus will be described in connection with the experiments.

Before drawing any conclusions, the numerical results were always plotted as curves.

E. Phenomena occurring during Perfusion with Sodium Chloride Solutions under Constant Conditions.

In the course of a prolonged perfusion with sodium chloride solution, the vein and ureter flow undergo certain changes which may be divided into four stages. These are seen with tolerable uniformity in almost every suitable experiment.

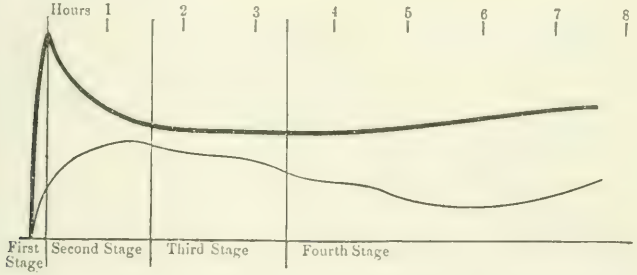


Figure 2. — The heavy line indicates the vein-flow, the light line the ureter flow during prolonged perfusion.

The *first stage*, following immediately on establishing the perfusion, consists in a rapid rise of the vein and ureter flow and of the oncometer. The vein flow usually reaches its maximum within fifteen minutes, but in rare cases this may not occur until thirty minutes. The ureter flow continues to increase for a much longer time. The period at which the oncometer reaches its maximum is variable.

The *second stage* is characterized by a diminution of the vein flow and a continued increase of the ureter flow. It begins about fifteen minutes after starting the perfusion and continues from one and one-half to two and one-half hours. The behavior of the oncometer is somewhat variable.

In the *third stage*, the curves for the vein and ureter flow are practically parallel and horizontal, with a slight tendency to fall. It begins in one and one-half to two and one-half hours, and lasts from three and one-half to four hours. This stage is evidently the most

favorable for delicate experiments. However, the changes in the other stages are so gradual and comparatively so small that they do not cause any serious interference.

The *fourth stage*, which lasts from three and one-half hours to four hours until the end of the experiments, *i. e.*, the fifth or sixth hour, shows a slight but definite increase of the vein flow. The ureter flow continues to fall during the early period of this stage, but later it shows a relative increase.

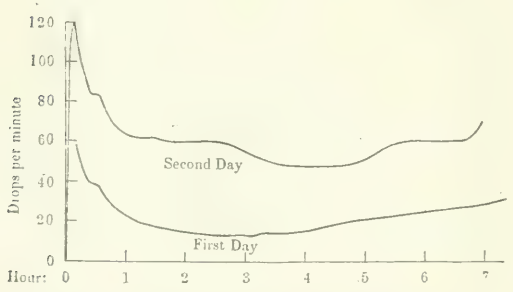


FIGURE 3. — Course of the perfusion on first and second day, on the same kidney (Experiment 60).

Repetition of the perfusion on the following day. — When the perfusion is repeated on the following day, the curve for the vein flow is practically repeated on every detail (Fig. 3). There is, however, only a very slight ureter flow, whilst the vein flow is relatively increased.

Discussion. — The fact that the phenomena of the vein flow are reproduced so faithfully on the second day, shows that their explanation must be mechanical, and that they are not due to a gradual death or drying of the kidney, nor to imbibition.

The general phenomena of the first stage are readily explained by the increase of the injection pressure. The rather long time required to reach the maximum of vein flow points to a very slow transmission of the injection pressure, or else to a gradual stretching of the vessels. The diminished vein flow in the second stage argues for the development of some obstruction. It occurs whether the injection pressure is high or low. It also occurs later in the course of the perfusion, whenever the pressure is raised (Experiment 92).

The oncometer does not show any considerable change during this stage. This indicates that the obstruction can be neither at the arterial nor at the venous end of the vessels, but that it must be located about the level of the efferent arteries or intertubular capillaries. Of these, the efferent arteries would be more likely to be affected. This would also explain the increased ureter flow which occurs during this stage, since the filtration pressure in the glomeruli would be raised. The assumption of an obstruction at this level agrees also with the fact that the oncometer

tends to increase during this stage when a high injection pressure (125 cm. to 140 cm.) is used, whereas it tends to fall with low injection pressure (60 cm.). With a pressure of 100 cm. it tends to remain constant.

This behavior of the oncometer also supports the view that the obstruction is developed by a pressure. No definite explanation of the nature of the obstruction can be given; but it appears reasonable to suppose that a continuous load gradually overcomes the elasticity of the vessels, and causes their displacement or mutual compression, as we shall see in the case of vein pressure.

The results are somewhat different when 2.5 per cent sodium chloride is used for the perfusion: In this case the vein and ureter flow and the oncometer all diminish, indicating that the obstruction develops very near to the arterial end. We shall show later that the concentrated salt solution causes dilatation of the renal vessels, and it is easily conceivable that the obstruction will occur in a different place in the dilated vessels than in constricted vessels.

The constant phenomena of the third stage indicate that the changes have reached a state of equilibrium. The gradual and progressive diminution of the ureter flow may be ascribed to *post-mortem coagulation* of the protoplasm. This is still more conspicuous on the following day. The slight increase of the vein flow in the fourth stage is probably due to a loss of the vitality of the vessels. This late *post-mortem dilatation* is shown very strikingly when the rate of the vein flow is compared after long intervals. When the perfusion was resumed in twenty-four to fifty hours after death, the vein flow was greater than that at the end of the first day's perfusions in every one of nineteen experiments, the mean increase being over three times, but going as high as twenty-nine times. In fifteen out of the nineteen experiments the flow on the second day was even greater (by a mean of one and one-half to two times) than the best flow on the first day. In two experiments the perfusion was repeated several times, showing that the vein flow increases with the time elapsing after death.

In Experiment 141, the initial flow was 140 drops per minute; after five hours, it had fallen to 125 drops; twenty-four hours after death, the perfusion was resumed; the rate was 300 drops. The perfusion was again resumed fifty hours after death, the rate being 400 drops. In Experiment 140, the rate was 170 drops twenty-four hours after death; 390 drops, twenty-nine hours after death; and 585 drops, fifty hours after death.

F. Conclusions.

1. Excised kidneys perfused with saline solutions under a moderate pressure yield a plentiful vein and ureter flow.

2. The ureter flow is not formed by rupture of the vessels.

3. Whilst the functions of the kidney are disturbed by excision, some degree of vital action on the part of the epithelium and of the vessels persists for something like twenty to forty hours after excision.

4. The formation of the ureter fluid during perfusion with saline solutions is mainly, if not entirely, due to filtration.

5. The circulation and filtration phenomena in the excised kidney undergo slow but distinct changes even when the conditions are kept constant.

These are explained by

(a) The gradual development of an obstruction to the circulation, occurring about the level of the efferent arteries.

(b) The post-mortem coagulation of the epithelium constituting the filtering membrane, introducing a gradually increasing resistance of filtration.

(c) A gradual post-mortem dilatation of the renal vessels.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. II.—THE EFFECTS OF CHANGES IN THE ARTERIAL VENOUS, AND URETER PRESSURE.

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A. Changes in the Arterial Pressure.

THESE may be readily produced in the kidneys prepared in the manner described in the preceding paper, by altering the level of the reservoir. The vein and ureter pressure was estimated by connecting the corresponding cannulas with upright glass tubes, and measuring the height to which the liquid rises.

For purely mechanical reasons, one would expect that the flow from the vein and ureter, the volume of the kidney, and the maximal pressure reached in the vein and ureter, should vary in the same direction as the injection pressure. Direct experiments confirm this presumption. However, the curves for all these factors are not strictly parallel. This may be seen from Fig. 1, in which the oncometer, vein flow, and ureter pressure are taken from Experiment 73, the ureter flow and the vein pressure being inserted from other experiments.

The ureter and the vein pressure trace a practically straight line, diverging from the pressure line as the pressure increases. The vein

flow, and to a lesser degree the ureter flow, form concave curves. The oncometer curve is convex. The phenomena are quite similar when the kidney vessels are dilated by hypertonic salt solution.

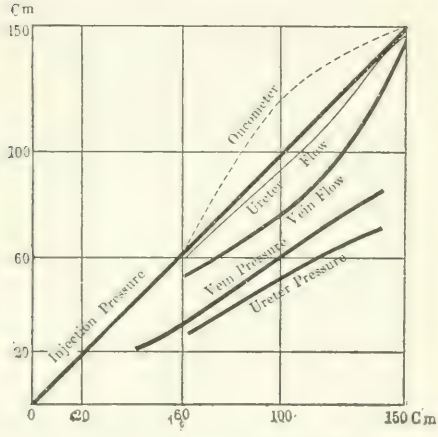


FIGURE 1. — Diagram of the effects of changes in the injection pressure.[1]

All these results can be explained by simple mechanical principles, and can be imitated on artificial models, as will be shown in the following:

1. The effect on the maximal vein pressure. — If the vessels of the kidney formed a closed system, the vein pressure should rise to exactly the level of the arterial pressure. In the actual experiments, it was found, however, that the maximal vein pressure is always lower than the arterial pressure, the degree of divergence increasing in a straight line with the injection pressure. This may be seen from Fig. 1, and from the following numerical data:[2]

Experiment 75. — 1 per cent sodium chloride solution.

Injection pressure.	Vein pressure.	Injection pressure.	Vein pressure.
cm.	cm.	cm.	cm.
40	17.5	120	71.0
60	28.0	140	85.0
80	40.0	120	70.0
100	56.0	80	37.0

The observed divergence could not be explained by obstructions in the vein; the continuance of the vein flow up to the maximal vein pressure shows that if any obstruction exists, it is only partial, and a partial obstruction would only delay, never prevent, the final level-

[1] In this and in the following diagrams, the arterial pressures are represented by the heavy, straight, ascending line. The vein and ureter flows are plotted on such a scale that the maximum flow coincides with the maximum injection pressure; the oncometer curve is represented in same manner. The curves for the vein and ureter pressure represent the actual values.

[2] We shall always limit ourselves to the reproduction of illustrative data, omitting the duplicate experiments which we have made to exclude accidental variations.

ling of the pressures. The only way in which the lower vein pressure can be explained is by a leak in the system. That such a leak exists, is shown by the slow stream of bubbles through the reservoir, occurring even when the renal vein is absolutely occluded (some 5 c.c. per minute). The leakage is not through the ureter, for the ureter flow is practically abolished by these high vein pressures; but an actual escape of fluid from the cortex of the kidney can always be noted. The vessels which leave the capsule of the kidney to form a collateral circulation are no doubt the anatomical seat of this leak.

The divergence can be imitated on an artificial model, constructed as in Fig. 2.

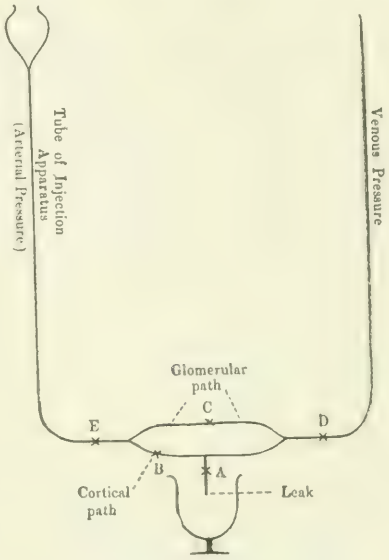


FIGURE 2. — Model for illustrating the effect of leakage. *X*, screw clamps for regulating the lumen of the tubes. (*A*), representing the leak; (*B*), representing the cortical path; (*C*), representing the path (glomerular) in which there is no leak;[1] (*D*), representing the vessels (veins) beyond the leak; (*E*), representing the vessels (arteries) proximal to leak.

Experiments with this model gave the following results: A divergence of the arterial and venous pressure, in the form of a straight line, was obtained whenever a leak was introduced into the system. Altering the conditions affected merely the degree of divergence, as follows:

a. The difference between the arterial and venous pressure increases,

(1) With the size of the leak.

(2) With the resistance central to the leak (at *E*).

These two are by far the most important factors.

b. The difference is also, but very slightly, increased by closing either of the capillary paths (*B* or *E*).

c. Partial obstruction of the vein causes a slight rise in the venous pressure.

[1] As the introduction of this path does not modify the phenomena, it was omitted in most experiments, the fluid going from *E* to *D* through *B* and *A*.

These data permit the quantitative reproduction of the condition in the kidney.

In the kidneys, the maximal vein pressure amounts to 70 cm. to 89 cm., with an injection pressure of 120 cm. The leakage (as measured by the bubbles in the reservoir) amounts to about 15 bubbles per minute. The size of the leak and of the arterial resistance of the artificial model can be readily adjusted so as to reproduce these figures. For instance: In Experiment 79 the clamps *B*, and *C* and *D* (Fig. 2) are left open, *A* and *E* are adjusted so that with the injection pressure at 125 cm. and the vein pressure at 62 cm. the reservoir shows 12 bubbles per minute.

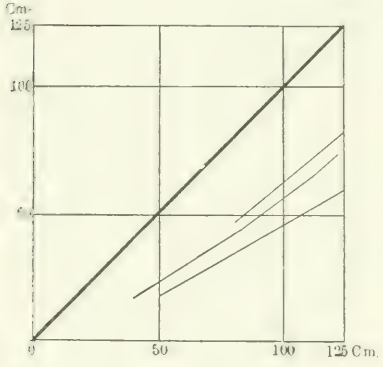


FIGURE 3. — Vein pressures observed on the model and on kidneys. The heavy line represents the injection pressure. The upper and lower fine lines represent the vein pressure on the artificial model, in Experiment 79; the middle line shows the vein pressure in the kidney, Experiment 74.

The results on varying the pressure (Fig. 3) concur quantitatively very closely with those seen on the kidney. The final pressures are reached with the same slowness as in the case of the kidney.[1] The agreement makes it sufficiently evident that we have reproduced the conditions in the kidney. For this it was necessary:

(a) To increase the arterial resistance, *i. e.*, central to the leak, so that it will only deliver 56 drops per minute, with full arterial pressure.

(b) To establish a leak, rather larger than the lumen of the artery (*i. e.*, delivering 125 drops per minute with full arterial pressure).

(The arterial pressure having been cut down in the model by the resistance, the leak only delivers 42 drops per minute)

The perfect agreement of the artificial model with the excised kidney permits us to apply the data learned in the former to the latter, as follows:

1. The principal resistance in the renal circulation is on the arterial side of the leak. In the perfusion of the excised kidney with 1 per cent sodium chloride solution, it is of such a degree that the fluid passed the resistance with a velocity of about 60 drops per minute.

2. The fact that the difference between the arterial and venous pressure is determined by two factors (resistance and leakage), which must naturally vary in different kidneys, explains why *the vein pressure for a given injection pressure is not* the same for different kidneys, *i. e.*, with 140 cm. injection pressure, the vein pressure in three different kidneys

[1] It takes about half an hour for the vein pressure to reach its maximum.

was 70.5, 85, and 87 cm. The last two figures are from the kidneys of the same animal, in which the anatomical conditions were naturally more uniform.

3. *Hyperisotonic solutions give a higher vein pressure:* With an injection pressure of 147 cm., the left kidney of the animal (Experiment 72), perfused with 1 per cent sodium chloride solution, gave a vein pressure of 70.5 cm.; the right kidney, perfused with 2 per cent sodium chloride solution, gave a vein pressure of 82 cm. The hyperisotonic solutions must therefore dilate the vessels on the arterial side of the leak (they may or may not alter those on the vein side).

4. Similar results are seen when the vessels are dilated with blood or with hydrocyanic acid. In Experiment 121, the following vein pressures were observed, with a constant injection pressure of 145 cm.

With 1 per cent sodium chloride	101.5 cm.
With dilute blood	115.0 cm.
With diluted blood to which hydrocyanic acid was added	117.2 cm.

Similar results were seen in Experiment 114.

2. The effect on the maximal ureter pressure. — The curve of the ureter pressure follows that of the vein pressure very closely, as shown in Fig. 1, and illustrated by the following numerical data, Experiment 72:

Injection pressure.	Ureter pressure.
cm.	cm.
60	38.5
100	67.0
125	82.0
147	97.0

This argues that the same mechanism is concerned in both cases. Indeed, the ureter system can be considered, in this connection, as a branch of the circulatory system, separated from the latter only by the filtration resistance. Since the ureter pressure is generally lower than the vein pressure we must suppose that the filtration occurs peripheral to the arterial resistance, — this of course agrees with the anatomic data. The ureter pressure may, therefore, be represented schematically as in Fig. 4. When the ureter and vein pressures are measured at the same time, they should approximate each other very closely, since we have seen that resistance peripheral to the leak has little effect.

This agrees with the actual results. In Experiment 73, the ureter

pressure was 70 cm. with the vein open. Measuring both pressures simultaneously, the vein rose to 82.5 cm., the ureter to 81 cm.

Since the vein flow is variable, the ureter pressure, like the vein pressure, is therefore, also variable in different animals, but agrees fairly well for the two kidneys of the same animal. Leaving the vein open amounts to the same thing as leakage, and the ureter pressure should, therefore, be considerably lower. This was observed uniformly. The difference should

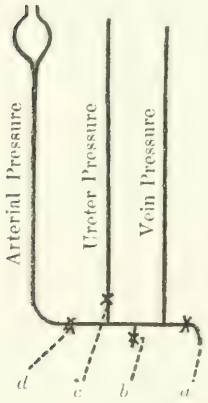


FIGURE 4. — Model to represent the ureter pressure. x = screw clamps; a = arterial resistance; b = resistance of filtration membrane; c = leak; d = vein.



FIGURE 5. — Diagram of the effect of the injection pressure on the vein flow. The straight line represents the increasing injection pressure; the upper curve, the vein flow in the kidney; the lower curve, the vein flow in the models.

increase with the rapidity of the vein flow. This was confirmed in Experiment 72, for hyperisotonic solutions (ureter pressure for 1 per cent sodium chloride solution, 97 cm.; for 2.5 per cent solution, 70 cm., with an injection pressure of 147 cm.). (It cannot, of course, be expected that the ureter pressure and vein flow in different kidneys should show any correspondence, for other factors (leakage, size of vessels, etc.) interfere too greatly.)

Hydrocyanic acid blood, on the other hand, gives a greater ureter pressure than blood alone, notwithstanding a very considerable increase of the vein flow. This can be explained on the assumption that the dilation is mainly on the arterial side of the leakage, an assumption which is confirmed by the pronounced increase of the volume of the kidney which occurs in these cases. I may cite Experiment 121.

	Ureter pressure.	Oncometer.
	cm.	cm.
Diluted blood	48.0	24.5
Hydrocyanic blood	76.5	27.3

3. The effect on the vein flow, oncometer, and ureter flow. — It may be seen by Fig. 1 that the vein flow is not directly proportional to the injection pressure, but that it forms a concave curve. Experiments with artificial models give precisely the opposite results, the curve being convex. Varying the resistance and the size of the leak merely alters the extent, not the direction of the curve. The difference may be represented diagrammatically as in Fig. 5. In other words, whilst the vein flow varies in the same general direction as the injection pressure, both in the kidney and in the models, the flow increases relatively more than the pressure in the excised kidney; whereas it increases relatively less in the model.

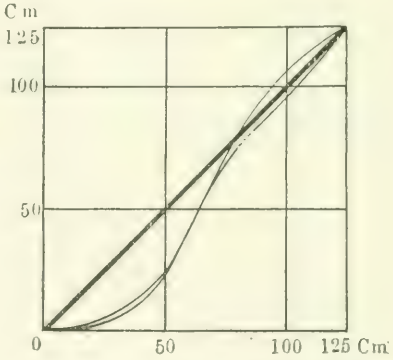


FIGURE 6. — Injection pressure on vein flow; curves obtained with "artificial glomerulus." (See Figure 11.)

The model result is easily understood; for the flow corresponds to the pressure, divided by friction; and since the friction increases with the velocity, the flow does not increase in direct proportion to the pressure. The same law must hold for the kidney. Since, however, the curve is precisely reversed in the case of this organ, another factor must come into play, which is not represented in the model. This factor is undoubtedly the distensibility of the renal vessels. In virtue of this, an increase of pressure *lessens* the friction, and the curve becomes concave.

This can also be imitated on an artificial model if an elastic resistance is employed. Fig. 6 shows the curves obtained with a model of this type. A reference to Fig. 1 will show the agreement in the direction of the curve.

That a dilation of the renal vessel actually occurs as the result of an increasing injection pressure is shown by the *oncometer* (Fig. 1).

The curve of the *ureter flow* follows the same general direction as

that of the vein flow. This indicates that the dilation occurs mainly on the arterial side of the glomeruli, just as one would expect.

B. Rhythmically Intermittent Injection Pressure.

The pressure in the renal artery being, under natural condition, intermittent, from the systole and diastole of the heart, it appeared interesting to investigate the effect of this intermittence. To produce an intermittent pressure, a side piece was intercalated between the reservoir and the kidney. The opening of this tube produces a prompt fall of pressure, comparable to the diastolic fall. The rhythmical contraction was then simulated by compressing the side tube with the fingers and releasing it, synchronously with the beats of a pendulum. This, it was expected, would much more closely resemble the natural intermittence of the arterial pressure, than simple closure of the injection tube; for on account of the great resistance in the kidney, the pressure in the renal artery falls but slowly with the latter manœuvre. The extent of the excursions could be readily regulated by varying the lumen of the side tube with a screw clamp. A mercury manometer was further introduced, showing the pressure changes. The arrangement is shown diagrammatically in Fig. 7. A manometer tracing showed that the systole and diastole were quite symmetrical; so that the mean pressure corresponded simply to the average of the maximum and mimimum pressure indicated by the manometer.

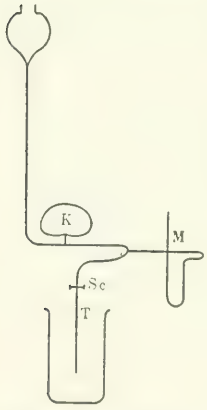


Figure 7. — Diagram of the apparatus for securing an intermittent pressure in the kidney. *M*, mercury manometer; *T*, tube, which was opened and compressed to produce the intermittence; *Sc*, screw cock; *K*, kidney.

Two series of experiments were made with this apparatus, the results agreeing so well that further trials seemed superfluous. We will omit the protocols of the experiments, stating only the conclusions:

In the excised kidney, perfused with 1 per cent sodium chloride solution, and for a given mean pressure, an intermittent injection pressure produces a better vein and ureter flow than does a constant pressure.

The flow varies on the same direction as the extent of the excursions.

An increased rate of intermittence increases the flow, and this may occur notwithstanding the lesser excursions.

Intermittent pressure also quickens the flow through a rubber tube, so that the effect may be accounted for by mechanical principles.

C. Obstruction of the Renal Vein.

1. Phenomena resulting from the absolute occlusion of the vein. — If the vein is clamped during the course of the perfusion, two striking phenomena occur very promptly: The kidney swells greatly, as can be seen by simple inspection, or by the oncometer; and the ureter flow is quickly reduced to a very small figure, but it never ceases completely. The phenomena are the same whether the vessels have been dilated by strong salt solution or not; but when strong solution is used, the ureter flow does not usually fall as low as with the weaker solutions. When the filtration is already very slow, on account of the coagulation of the epithelium, obstruction of the vein has but little further effect on the ureter flow.

A few figures will sufficiently illustrate the very uniform results:

	Ureter flow with vein		Oncometer with vein	
	Open.	Clamped.	Open.	Clamped.
Fresh kidneys perfused with 1 per cent sodium chloride solution.				
Experiment 32	12.5	2.5		
Experiment 69	16.0	6.0	22.6	25.4
Fresh kidneys perfused with 2.5 per cent sodium chloride solution.				
Experiment 71	28.0	15.0	9.0	22.5
Experiment 73	24.0	5.0	18.0	23.5
Old kidneys.				
Experiment 36. Kidney has lain for 3 days	3.5	4.0		
Experiment 37. Perfused with 80% alcohol, then left over night	3.0	2.0		

These results agree entirely with those which Ludwig[1] obtained by a similar experimental arrangement. (Ludwig employed an excised kidney perfused with a solution containing 3 per cent of gum arabic and 1 per cent of sodium chloride, under a water pressure of 1 m.)

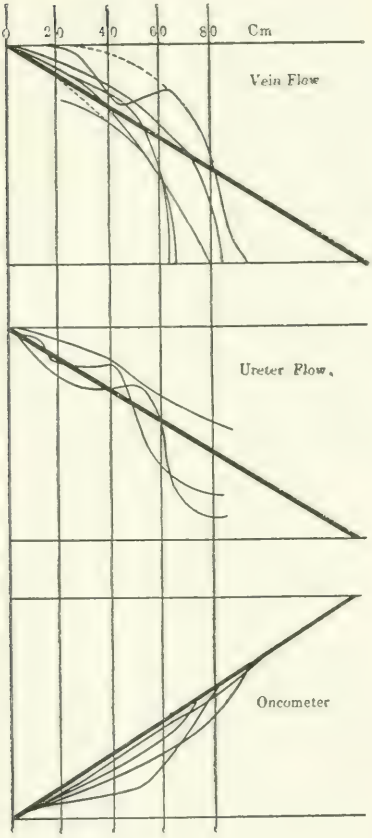


FIGURE 8. — Effect of vein pressure. The curves of the different experiments are superimposed. The straight line drawn from 0 to 145 cm. (the mean injection pressure) represents the increase of vein pressure (a fall in this line indicating an increase of vein pressure, in the case of the vein and ureter flow).

2. Phenomena resulting from the graduated increase of vein pressure. — If the pressure in the renal vein is increased by raising the out-flow tube to the desired height, it is found that, as the vein pressure is made to rise, the flow from the vein and from the ureter is diminished, whilst the oncometer shows an increase of volume. The curves of flow are not, however, parallel to the pressure (see Fig. 8). It may be remarked that vein pressures up to 40 cm. have a relatively small effect on the flow, and that between 40 cm. and 80 cm. the flow from both vein and ureter is suddenly diminished. The curve is very much the same whether 1 per cent or 2.5 per cent sodium chloride solution is used.

3. The effect of vein pressure on vein flow in artificial models. — To explain the peculiar curves, the flow from artificial models was investigated. The results were as follows (Fig. 9):

(a) When the vein pressure is increased in a simple, non-leaking model, the curve is indeed convex, but it is perfectly regular.

(b) When a leak is introduced into the system similar to that in

[1] LUDWIG: Cited from HENLE and MEISSNER'S Berichte, 1863, pp. 323 to 328.

the kidney[1] the flow lessens relatively more, as the vein pressure is increased, reproducing the kidney curve.

(c) The phenomenon can be reproduced in a non-leaking system, and even more strikingly when the model is composed of elastic tubes capable of mutual compression, such as will be described later. Similar curves were obtained with the "artificial glomerulus" (Fig. 11). They could doubtless be reproduced also with the twisting valve to be described later.

4. The cause of the vein flow curve. — The characteristic peculiarity of the curve of the excised kidney, the relatively small reduction of the vein flow by low vein pressures, and the rather suddenly larger reduction at higher pressure can therefore be reproduced in two ways:

(a) By introducing a leak in the system.

(b) By arranging the tubes so that they will obstruct each other as the vein pressure increases.

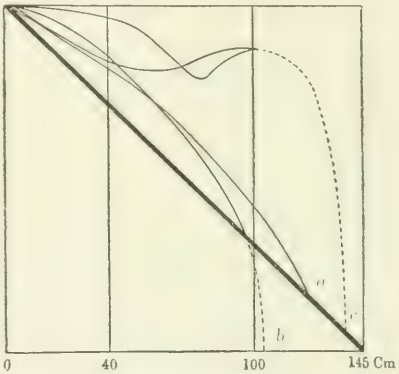


FIGURE 9. — Effect of vein pressure on the vein flow in artificial models. (*a*), non-leaking model; (*b*), leaking model; (*c*), model with mutually compressing tubes, no leak (see Fig. 10).

The first of these conditions (leakage) is of course present in the kidney. We shall see that the second conditions (mutual compression of the vessels on raising the vein pressure) exists also. Indeed, it would seem that the latter is the more important factor, for the volume of the kidney increases with the sudden drop of vein flow; this is just what would occur if an obstruction developed; whereas, if the diminution of vein flow were due to leakage, the oncometer would fall.

5. The cause of the diminished ureter flow. — *Pressure on the vein* — no matter how small — *causes a diminution of the ureter flow* which progresses as the pressure increases (Fig. 8) the ureter flow reaching its lowest level when the vein is entirely occluded. There is, even at that time, some flow, — very little if 1 per cent sodium chloride is circulated; rather more if stronger solutions are employed. Com-

[1] Injection pressure, 125, vein pressure, 90; 25 bubbles per minute in reservoir; see Fig. 2.

plete arrest can only be obtained by a further increase of vein pressure, *i. e.*, by injecting the kidney through the renal vein. *In not a single instance did a rise of vein pressure lead to increased ureter flow.*

As has been repeatedly demonstrated in the preceding pages, the formation of the ureter fluid obtained in these experiments is a plain filtration process. As such, the rapidity of its flow should be proportional to the filtration pressure. It might be argued that an increase of vein pressure should raise the filtration pressure and this increase the ureter flow. To do so would be to forget that the filtration pressure is the *difference* between the pressures on the two sides of the filtering surface. To increase the vein pressure will undoubtedly increase the pressure on the one side — that of the vessels ; but it would only increase the filtration if it did not also increase the pressure on the side of the tubules; and if it did not diminish the extent of the filtering surface. The very fact that the ureter flow is diminished by increasing the vein pressure suffices to show that one or the other process, or both, occur (changes in the nature (permeability) of the filtering surface may be excluded).

Ludwig,[1] who was the first to investigate this problem, concluded from histological evidence that the swelling of the veins consequent on increased vein pressure compressed the fine tubules in the boundary layer, thereby raising the ureter pressure. These findings have never, as far as I know, been contradicted.

In addition to this, it is, however, quite possible that another mechanism comes into play ; it is conceivable that the increased venous pressure causes the glomeruli to swell so as to fill Bowman's capsule, diminishing the filtering surface to practically nothing. There is no direct proof for this hypothesis, but it is suggestive that Hellin and Spiro[2] found that diuresis (caffein and phlorhizin) is prevented by those renal poisons (arsenic, cantharidin) which cause a swelling of the glomerular tufts with obliteration of Bowman's capsule.

D. Injection of the Kidney through the Vein.

1. Experimental results. — The phenomena described for occlusion of the vein — the swelling of the kidney and the diminution of the

[1] Ludwig, C.: Sitzungsbericht der kaiserlichen Akademie der Wissenschaften zu Wien, Nov., 1863, xlviii. Quoted by Meissner in Henle's Berichte, 1863, p. 324.

[2] Hellin, D., and Spiro, K.: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xxxviii, p. 368.

ureter flow — occur still more strikingly if the injection is attempted through the renal vein. Only rare drops flow from the artery, even when the vessels are dilated by strong salt solution; the urine flow is even smaller than when the veins are compressed; the anuria occurs also with strong salt solution; and the kidneys swell more than when the vein is occluded. The previous conditions are not restored at once when the injection is changed back to the artery. The following experiments will illustrate these statements:

PROTOCOLS.

No. of exp.	Ureter flow with				Oncometer with			
	Per cent of NaCl.	Arterial injection.	Vein injection.	Clamped vein.	Per cent of NaCl.	Arterial injection.	Vein injection.	Clamped vein.
85	1	37	0	$\frac{1}{2}$	1	19.0	21.2	20.8
"	2	76	$\frac{1}{2}$	20	2	18.8	21.0	21.0

2. The cause of the anuria is undoubtedly the same as with vein occlusion; but since the vein pressure rises even higher when the injection is made by the vein, the anuria is even more complete, and is almost absolute even when 2 per cent salt solution is used.

3. Why cannot the kidney be perfused from the vein? —The experiments just described suffice to show that the kidney vessels are practically impermeable to injections made through the vein. Although I was not aware of the fact when I started my experiments, this has been the common experience of all experimenters.

Ludwig[1] refers to it and suggests an explanation; Lindemann[2] credits the experiment to Heidenhain and confirms it; De Souza[3] mentions it as a well-known fact. All these authors state that the injection cannot be forced into the glomeruli. The great swelling which I have noticed, and which even surpasses that on occluding the vein, shows that the obstruction is situated toward the arterial end, *i. e.*, about the efferent artery or the efferent portion of the glomerular capillaries.

[1] Ludwig, C.: Sitzungsbericht der kaiserlichen Akademie der Wissenschaften zu Wien, 1863, xlviii. Quoted by Meissner in Henle's Berichte, p. 323.

[2] Lindemann, W.: Zeitschrift für klinische Medicin, 1898, xxxiv, p. 299.

[3] De Souza, D. H.: Journal of physiology, 1901, xxvi, p. 149.

It is generally conceded that the renal veins are devoid of valves; nevertheless, the phenomena which we have just described show a very efficient valvular mechanism. How is this functional valve to be explained in the absence of the ordinary anatomical valves?

Ludwig[1] advanced an explanation which is probably responsible for at least a large part of the phenomenon. The original paper is not accessible to us. As quoted by Meissner, it suggests that the arrangement of the vessels in the glomeruli is such that the venous portion of the vessels can compress the arterial portion, but not the converse. The statement that a communicating system of tubes can occlude itself by mutual compression seemed to me to merit experimental confirmation. I have, accordingly, constructed some models which reproduce the phenomena very well. The necessary conditions are: an inelastic capsule (corresponding to the capsule of the glomerulus), enclosing a system of distensible and compressible tubes of different surface area. In such a model, the fluid flows readily, if the injection is made in the direction of the tube having the smaller surface to that of the larger surface, but practically no flow occurs when the injection is attempted in the opposite direction, because the swelling of the tubes of larger surface obliterates the lumen of the smaller tubes more or less completely.

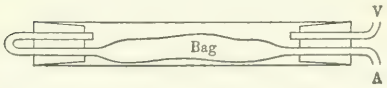


FIGURE 10. — Balloon-valve. (*A*), corresponds to the arterial; (*V*), to the venous end of the renal circulation.

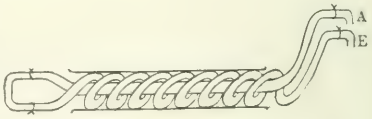


FIGURE 11. — "Artificial glomerulus," constructed from dog's intestine (see text).

One model of this type is illustrated in Fig. 10. A very thin collapsible rubber tube (an elongated toy balloon) is introduced into a glass tube and connected as shown in the figure. In this particular model, an injection pressure of 125 cm., when connected with the end *A*, gave an outflow of 38 c.c. per minute; when connected with *V*, only ten drops escaped per minute (Experiment 83).

It is possible to construct a model which approximates the conditions which may be supposed to exist in the glomerulus so closely that I feel tempted to call it an "artificial glomerulus." It is formed from dog's intestine, by winding a long piece spirally around a shorter straight piece

[1] LUDWIG: *Loc. cit.*

(see Fig. 11). All kinking must be carefully avoided, and for this reason the two ends are connected by a U-shaped glass tube. The spiral is then introduced into a glass tube of such dimension that it is filled when the intestine is moderately distended, but so that it prevents maximal distension. The free ends of the intestine are tied on glass cannulas which are supported on stands.

The intestine represents the vessels of the glomerulus: *A*, the afferent artery, *E*, the efferent artery. The glass tube serves as Bowman's capsule, rendered unyielding by the surrounding tubular tissue.

In this model (Experiment 87), with an injection pressure of 125 cm. the outflow from *E* was 25 c.c. in nine seconds, when *A* was connected with the injection; if the injection was attempted through *E*, absolutely no fluid flowed from *A*.

(This model also served to illustrate the effect of injection pressure and of vein pressure on vein flow, as described previously. It reproduces also another interesting elasticity phenomenon: In working with excised kidneys, it is sometimes observed that the vein flow is rhythmical, a period of rapid flow alternating regularly with a period of slow flow. This phenomenon was observed with the artificial glomerulus in Experiment 87: with the vein pressure at 0, the flow occurred in spurts, separated by regular intervals; these intervals were longest with low injection pressures, and shortened as the pressure was increased; but persisted with an arterial pressure of 125 cm. They disappeared, however, when the vein pressure was raised to 25 cm. or higher, to reappear when it was again lowered to 0.)

Mutual compression is not, however, the only way in which collapsible tubes may assume a valvular function. It is only necessary to make a few twists in a free-lying coil of intestine, and then attempt injection from either end, to see that, without any compression, a valve action is established by kinking. In a free twist of this kind, injection from one end gave an outflow of 25 c.c. in eight seconds; from the other end, one hundred and fifty seconds were required to collect 25 c.c. In order to have this phenomenon, the vessels must be fairly movable, a condition which seems to exist at least to some degree in the glomerulus.

The conditions for a valve action by either mutual compression or twisting would therefore seem to be best realized in the glomeruli, and there can be very little doubt that the obstruction is located in them. Which of the two processes is most concerned in the phenomenon can perhaps be decided by histologic examinations.

I may add that the illustrations of Johnston's reconstructed glomerulus[1] seem to show that the afferent arteries go into the midst of the glomerulus — a condition very favorable to compression.[2] His illustrations also show a twist in the vessels which would be peculiarly favorable to a kinking valve; so also would the sharp angle at which the vessels connect with the glomerulus (Virchow).

E. Effects of Increasing the Ureter Pressure.

The ureter pressure in these experiments was adjusted at any desired height by connecting the ureter cannula with a rubber tube, the delivery end of which was placed at the desired level. The results are shown in Fig. 12. They consist in the diminution of the ureter flow, a lesser diminution of the vein flow, and a slight increase in the oncometer. The flow and oncometer do not return to normal immediately after removing the pressure, just as in the case of vein pressure.

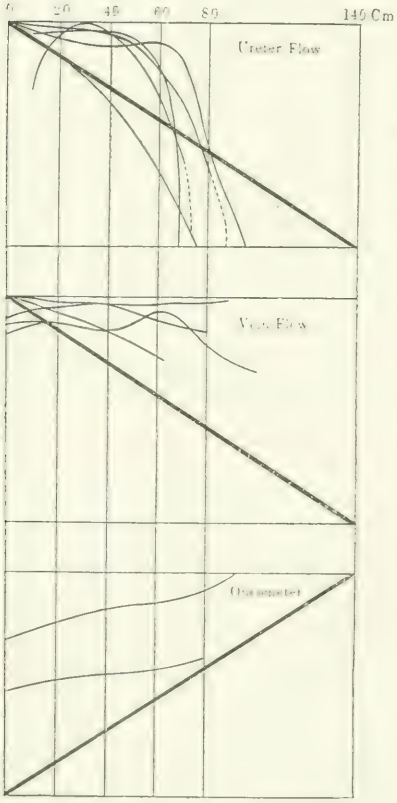


Figure 12. — The effects of ureter pressure (see Fig. 8 for explanation).

The slight diminution of the vein flow is explained, according to Hermann,[3] by the pressure of distended urinary tubules on the veins. It would seem, from one experiment, that the effect on the vein is less when the vessels are dilated by strong salt solution.

The effect on the oncometer is explained by the stasis of the fluid in the tubules and in the vessels.

The effects of ureter pressure on the ureter flow are entirely parallel to the effects of vein pressure on vein flow. In both cases small pressures — to 40 cm. or 60 cm. — have

[1] Johnston, W. B.: Johns Hopkins Hospital bulletin, 1900, xi, p. 24.

[2] According to Ludwig, however, the afferent vessels are situated toward the periphery of the glomerulus, the efferent toward the centre. (Quoted from Heidenhain: *Loc. cit.*, p. 315.)

[3] Hermann, Max: Sitzungsberichte der Wiener Academie, 1861, xlv, p.

comparatively little effect, a sudden diminution occurring above these pressures.

In connection with the study of vein pressure, we found that the curve might be due either to increased leakage, or to obstruction in the tubule. The only leakage which could occur in the case of the ureter pressure would be by way of the veins; but the curves show that the vein flow is not increased; and the absence of leakage is also confirmed by the steady rise of the oncometer. It would appear, therefore, *that ureter pressures above a certain point cause an obstruction of the urinary tubules*, probably by mutual compression. The apposition of the thin and heavy portions of the tubules in the boundary layer would furnish the necessary conditions.

The ureter flow is not increased when the ureter pressure is raised intermittently. This was confirmed by experiments on three kidneys.

In Experiment 130, the pressure was raised to 45 cm. or 55 cm. three times in fifty minutes; the ureter flow was 2.8 c.c. per fifteen minutes, whereas it amounted to 4 c.c. when the ureter was left permanently open. Similar results were obtained on repeating the experiment.

In Experiment 127, the ureter pressure was raised to 30 cm. twenty-seven times in fifteen minutes. The mean ureter flow was 5 c.c. per minute; with the ureter open, the mean flow was 5 c.c. per minute before the intermittent pressure was applied, and 4.6 c.c. after.

In Experiment 121, the ureter pressure was raised to 20 cm. or 25 cm. six times in 18 minutes, giving a mean ureter flow of 0.8 c.c. per minute. With the ureter open, the mean flow was 0.83 c.c. per minute.

F. Bearing of the Results obtained with Excised Kidney on the Conditions in the Living Body.

We would repeat that our object in these investigations has been to learn in how far the phenomena occurring in the living body could be explained by mechanical causes. This we have attempted to do by reducing the kidney to a condition in which vital actions are almost or completely eliminated, as far as the present phenomena are concerned. A comparison of the results is therefore necessary.

1. The effects of arterial pressure. — The dependence of vein flow and of the volume of the kidney on the arterial pressure follows so obviously and necessarily from mechanical laws, in the body as well as

345. Quoted from Heidenhain's article in the fifth volume of Hermann's Handbuch, p. 317.

without, that it seems superfluous to quote any experiments in their confirmation. (As regards the oncometer correspondence, we may refer to the results of Cohnheim and Roy).[1] A corresponding effect on the ureter flow is an equally necessary deduction, if the separation of the urine in the glomeruli is conceived as a filtration process. The experimental evidence is unanimous that a correspondence actually exists in the body. This, as well as the correspondence of the ureter pressure, is well shown by the curve of Experiment 12, of Gottlieb and Magnus.[2]

The increased ureter flow was attributed by Ludwig to filtration, whereas Heidenhain denied this explanation, and referred it to a stimulation of the vital secretory mechanism. Our experiments show at least that the observed phenomena would occur as assumed by Ludwig, *i. e.*, by pure filtration. They do not, of course, prove that a vital action does not take place in addition, and if so, which is the more important.

2. The better circulation with rhythmical injection pressure has been noted by many investigators (*e. g.* Brodie[3]) with many different organs. Its cause is shown to be purely mechanical. It is interesting to note that the ureter flow is also increased mechanically.

3. The maximal ureter pressure. — The maximal ureter pressure in the body varies considerably in different animals. For arterial pressures of about 100 mm. of mercury (130 cm. water), the ureter pressure lies between 30 mm. and 66 mm. of mercury (39 cm. to 88 cm. water); the original result of Hermann, 60 mm. (78 cm. water), seems to be a fair mean. The corresponding ureter pressure obtained by us varied between 70 cm. and 95 cm. water. A very close similarity exists therefore between the ureter pressure in the body and in the excised kidney. The conditions are, however, so different in the two cases, that the close correspondence must be accidental. Another similarity exists between the lowest blood pressure at which any urine is secreted. In the living body this corresponds to about 30 mm. to 50 mm. of mercury (39 cm. to 65 cm. water); in an excised kidney, to 30 cm. to 70 cm. of water. This correspondence must also be accidental.

The correspondence between the effects of various experimental

[1] Cohnheim and Roy: Virchow's Archiv, 1883, xcii, p. 424.

[2] Gottlieb and Magnus: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlv, p. 253.

[3] Brodie, F. G.: Journal of physiology, 1903, xxix, p. 271.

conditions is of much greater importance. In the body, as in the excised kidney, the maximal ureter pressure varies with the arterial pressure.

The curve of Experiment XII of Gottlieb and Magnus[1] shows the same straight divergence, with increasing pressure, which is shown in Fig. 1. These facts, however, are compatible with both the filtration and the secretion theories. Gottlieb and Magnus[2] have found that saline diuretics may increase the ureter pressure independent of the blood pressure, and that the passing off of the diuresis causes a fall of ureter pressure, again independent of the blood pressure. This they use as an argument against the filtration theory. In doing so, they lose sight of one important factor, viz., that the hydræmia, by lessening the viscidity of the blood, increases the glomerular pressure without a corresponding change in the general blood pressure. This is shown by our Experiment 121: with sodium chloride solution, the ureter pressure was 75 cm.; with diluted blood, only 48 cm. The same phenomenon occurs when the afferent vessels are dilated by hydrocyanic acid. That a corresponding increase of glomerular pressure occurs in the body is shown by the increase of the oncometer during hydræmic diuresis.

The values of the ureter pressure have a further significance: If our deductions are correct, they correspond to the values of the glomerular pressure. In the dead kidney, therefore, an arterial pressure of 125 cm. to 140 cm. (of water) is reduced at the level of urine formation to 70–91 cm. (54 mm. to 70 mm. mercury). This result cannot, of course, be applied directly to the body, for in the latter the conditions are altered by the dilation of the vessels and by the viscidity of the blood; but there is no reason to suppose that the reduction is very different in the intact kidney.

4. The effects of vein pressure on the kidney. — The effects of compression of the renal vein has been quite extensively studied.

The early literature is summarized by J. Paneth.[3] The first experimenters, beginning with Robinson (1842), confined their attention only to the speedy death, the albumin, casts, and blood in the urine. H. Meyer[4] (1844) seems to have been the first to mention

[1] Gottlieb and Magnus: *Loc. cit.*

[2] Gottlieb and Magnus: *Loc. cit.*, p. 251.

[3] Paneth, J.: Archiv für die gesammte Physiologie, 1886, xxxix, p. 515.

[4] Meyer, H.: (quoted from Heidenhain: *Loc. cit.*, p. 325).

the diminution of the urine. Ludwig,[1] in 1863, reproduced it on the excised kidney perfused with saline solution, agreeing entirely with our results. If we are justified in assuming that the urine formation in these excised kidneys is a pure filtration process, every ground is removed from those arguments against the filtration theory which are based on the anuria following the vein occlusion. Indeed, we cannot see how any cause other than filtration could be made to explain the phenomenon in the salt-perfused kidney. It is astonishing that this convincing experiment of Ludwig's should have been almost forgotten. Even where it is quoted, it is not given the weight it deserves. Heidenhain describes it on p. 317 of his article, but seems to have lost sight of it in his arguments on p. 325. In their recent epitome, Spiro and Vogt,[2] also after citing this very experiment, state that the anuria is probably due to the disappearance of urinary substances from the stagnating blood.

Some excuse for this exists, however, in the entirely anomalous results of Schwarz and of Kobert, to be mentioned later.

From 1863 till 1896 all experimenters found that compression of the renal vein diminished the flow of urine. Of these, only the paper by Paneth[3] requires especial mention. He secured increase of pressure in the renal vein, with a minimum disturbance, by compressing the vena cava with a weighted ligature. This results in diminished urine flow, in every case, and with the smallest effective pressure (4.5 cm. to 7.3 cm. of water). This agrees with our results on the dead kidney.

He also found that the flow remains small for some time after removing the load. This also occurs in our experiments; I am inclined to attribute it to twisting of the vessels in the glomeruli. Experiment 73, (2.5 per cent sodium chloride solution) is an example of this after-effect (see page 273).

Paneth then puts the question whether such small pressure as 4.5 cm. to 7.3 cm. of water could produce diminished ureter flow by mechanical causes, *i. e.*, by compression of the tubules. I would recall that in our kidneys very small vein pressures produce a distinct diminution of ureter flow, so that mechanical causes must suffice for the purpose; whether they are sufficient to explain Paneth's results quantitatively, is difficult to decide. Paneth himself answers the question in

[1] Ludwig: *Loc. cit.*

[2] Spiro and Vogt: Ergebnisse der Physiologie, 1902, i, p. 423.

[3] Paneth: *Loc. cit.*

the negative, because the injection of sodium nitrate increased the urine when it had been completely stopped by compression of the renal vein. Our results remove the force of this argument, for our kidneys also give a greater diuresis with strong salt-solution[1] than with weak, after occlusion of the vein:

Experiment 73.

Increasing Vein Pressure.				Decreasing Vein Pressure, after a Maximal Pressure of 82.5 cm.		
Vein pressure.	Ureter flow.	Oncometer.	Vein flow.	Ureter flow.	Oncometer.	Vein flow.
0	33	17.9	32.0	24	16.8	17.0
40	20	20.5	17.0	18	21.0	11.5
60	7	21.6	10.0	5	22.5	
70	6	22.5	5.2	5	23.0	2.2

Ureter flow (after occlusion of vein) with 1 per cent sodium chloride, and 2 per cent sodium chloride:

Drops per minute	1 per cent NaCl.	2 per cent NaCl.
Experiment 84	0	13
" 85	$\frac{1}{3}$	20

The explanation of this phenomenon is, therefore, mechanical, and, I believe, quite simple; as I shall show in a later paper, the hyperisotonic solutions cause a shrinkage of the kidney cells, and thereby widen the lumen of the tubules, so that a given rise of vein pressure causes less obstruction. The ureter flow stops even with the stronger solution, if only the vein pressure is raised sufficiently, by injecting through the vein.

We see, therefore, that the results of Paneth are, at least qualitatively, identical with those obtained from dead kidneys, and that they cannot, therefore, be used to support the vitalistic theory.

Of other papers of this period, I need only refer to that of Munk and Senator,[2] who found anuria on compression of the renal vein in excised kidneys perfused with defibrinated blood.

[1] Paneth injects 25 per cent sodium nitrate, *i. e.*, strongly hyperisotonic.

[2] Munk, I., and Senator: Archiv für pathologische Anatomie, 1888, cxiv, p. 1.

So far, then, all experimenters had seen the urine diminished on compressing the renal vein; but in 1900 there appeared a paper by Schwarz,[1] which threatened to overturn all the previous work. Schwarz worked with dogs whose blood had been defibrinated by Pick's method. In these, he connected the renal vein through tubing with the femoral vein. The tubing bore a side piece through which the outflow of blood from the kidney could be measured. A clamp permitted the graduated compression of the tube, and hence of the renal vein. With this admirable experimental arrangement he found that, in ten animals out of thirteen, compression of the renal vein *increased* the urine flow. In the remaining three dogs, the orthodox diminution of ureter flow was obtained. At least one of these showed clotting in the renal vein. Schwarz, therefore, attributes the uniform results of his predecessors to intravascular clotting. In the dogs which showed diminution, the very first result of clamping the vein was also an increased urine flow. (This I have observed on the dead kidney; it is simply due to the squeezing out of the contents of the tubules by the swelling veins.)

Schwarz supports his results by quoting from Tammann.[2] According to the latter, Kobert, perfusing ox's kidney with saline mixtures, obtained two to ten times as much ureter filtrate after clamping the renal vein, *i. e.*, just the reverse from my results.

Kobert's results not being accessible to me, I will refrain from criticism, and only repeat that in not a single case, out of fourteen experiments, under the greatest variety of experimental conditions, did I obtain any increase of ureter filtrate.

Schwarz's results are flatly contradicted by De Souza.[3] The latter finds that ligation of the renal vein is not followed by intravascular clotting within the limits of an ordinary experiment, and that obstruction of the renal vein, partial or complete, causes diminution or cessation of the urine, whether the blood is defibrinated or not. He also explains the brief increase of urine flow by expulsion of the (pelvic) urine. He can offer no explanation of Schwarz's astonishing results. I am equally at a loss, after careful study of his paper. One is tempted to think that he obstructed his ureters. But the persistence of his increased urine flow does not accord with this error, which, besides, could scarcely escape an experienced operator.

We are, therefore, obliged to leave his results entirely unexplained, but opposed by the experience of practically every other investigator.

[1] Schwarz, L.: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliii, p. 1.

[2] Tammann, G.: Zeitschrift für physikalische Chemie, 1896, p. 180.

[3] De Souza, D. H.: Journal of physiology, 1901, xxvi, p. 139.

5. The effects of ureter pressure on the kidney. — Hermann [1] placing the ureter of a narcotized dog under a pressure of 35 mm. mercury (45 cm. water), found a decrease of the vein flow, and attributes this to a compression of the veins in the medulla by the distended urinary tubules. These observations are extended by Lindemann,[2] who finds that ureter pressures to 50 mm. mercury (65 cm. water) diminish the vein flow but little (some 10 per cent), whereas larger pressures (100–200 mm. mercury, 130–260 cm. water) lessen the vein flow very considerably; by the oncometer he shows that this is due to compression, first, of the veins, then, of the arteries. These results on the living kidney agree, therefore, with those obtained by me on the dead kidney, as far as concerns the vein flow and oncometer.

In regard to the effect on the ureter flow, the observations on animals must be divided into two classes, according to whether small or large ureter pressures are used, for the two apparently give opposite results.

Small ureter pressures. — A. Steyrer [3] reports three cases of unilateral compression of the ureter, resulting in an increased quantity of urine, with lessened specific gravity, freezing point, and organic and inorganic substances. Pfaundler [4] reports increased quantity of urine in three dogs and one woman. Schwarz [5] finds that a ureter pressure of 10 cm. to 25 cm. of oil increases the urine-flow, whilst *larger pressures* diminish it. Cushny [6] found the urine in rabbits always diminished by ureter pressures of 15 mm. of mercury (19.5 cm. of water).

The diminished ureter flow is entirely in accord with my experiments. The increased flow seen by Pfaundler, Steyrer, and Schwarz, I have not observed (except in one instance, in which it was very probably accidental, and due to the removal of an ureter obstruction). I am, therefore, inclined to attribute this increase to causes (vital?) which do not exist in the excised kidney.

[1] Hermann, Max: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften zu Wien, 1861, xlv, p. 345. Quoted from Heidenhain's article in the fifth volume of Hermann's Handbuch, p. 317.

[2] Lindemann, W.: Beiträge zur pathologischen Anatomie und Physiologie, 1897, xxi, p. 500, and Zeitschrift für klinische Medicin, 1898, xxxiv, p. 305.

[3] Steyrer, A.: Beiträge zur chemischen Physiologie und Pathologie, 1902, ii, p. 312.

[4] Pfaundler, M.: Hofmeister's Beiträge, 1902, ii, 336.

[5] Schwarz, L.: Centralblatt für Physiologie, 1902, xvi, p. 281.

[6] Cushny, A.: Journal of physiology, 1902, xxviii, p. 431.

In a recent paper, Filehne and Ruschhaupt[1] suggest that the increased ureter flow observed by Pfaundler, Schwarz, and Lindemann, was mechanical and due to the intermittent raising and lowering of the ureter pressure. The data on page 269 show that this has no effect on the dead kidney, and the phenomenon is, therefore, not mechanical.

G. Conclusions.

In excised kidneys, perfused with saline solutions, the following results were obtained:

1. **Effects of the injection pressure.** — The vein flow, ureter flow, oncometer, maximal vein pressure, and maximal ureter pressure, all vary in the same direction as the injection pressure. When represented in the form of curves, it is seen that they are not parallel to the latter. All the variations can be reproduced on artificial schemata.

The maximal vein pressures and ureter pressures form a straight line, which diverges below the injection pressure. This divergence is due to a leakage through the collateral vessels. The ureter pressure is practically equal to the circulatory pressure in the glomeruli, which can be estimated by this means (in the dead kidney). The vein flow forms a concave curve. This is due to the widening of the renal vessels by the pressure. The ureter flow also describes a somewhat concave curve, whilst that of the oncometer is convex.

2. **Rhythmically intermittent pressure** gives a better vein and ureter flow than the same mean constant pressure. The increase varies in the same direction as the rate and amplitude of the excursions. Rhythmic pressure has a very similar effect on the outflow from a tube.

3. **Obstruction of the vein** causes a swelling of the kidney and an almost complete abolition of the ureter flow.

Graduated increase of vein pressure causes a diminution of vein and ureter flow and swelling of the kidney. The effects are comparatively slight with pressures to 40 cm. or 60 cm. of water, when they suddenly increase. Similar curves can be obtained in models containing a leak, or those in which increased vein pressure leads to obstruction of the tubes by mutual pressure (or twisting). The oncometer shows that in the kidney the curve of vein flow is produced by obstruction. The diminution of the ureter flow is due to

[1] Filehne and Ruschhaupt: Archiv für die gesammte Physiologie, 1903, xcv, p. 209.

compression of the urinary tubules, and perhaps also to obliteration of the space of Bowman's capsule. Measures which dilate the urinary tubules therefore counteract the anuria of vein compression ; but the anuria occurs also in this case, if the vein pressure is sufficiently raised.

4. There is a close qualitative *correspondence between the phenomena observed in the living kidneys in the body, and the mechanical filtration phenomena in the excised kidney.* This does not by any means exclude the co-operation of "vital" forces in the body. If such are at work, they must, however, either act in the same direction as the filtration phenomena, or else they must be of very subsidiary importance. If filtration and vital actions both tend in the same direction, no conclusion as to their relative importance can be drawn from the present experiments.

The experiments refute three of the objections of Heidenhain to the filtration theory,[1] viz. (according to Heidenhain) :

(1) It is doubtful (from analogy) whether increased arterial pressure can cause increased filtration through the capillaries.

(2) The epithelium of the glomerular capsule would increase the filtration resistance to a practically unsurmountable degree.

(3) According to the filtration theory, venous pressure must increase the filtration.

(A further objection (8), that the filtration theory cannot explain the saline diuresis, will be refuted in Papers III and VI.)

[1] Hermann's Handbuch der Physiologie, 1883, v, pp. 360 and 361.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. III.—ANISOTONIC SOLUTIONS.

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IN a previous paper,[1] I have pointed out that the vein and ureter flow in excised kidneys varies in the same direction as the molecular concentration of the perfusing fluid, that this effect is very prompt and very large, and that it occurs also in dead kidneys. These results have been fully confirmed by my subsequent experiments. In this paper I need, therefore, give merely some additional details, and the effects on the volume of the kidney.

A. The Effect on the Vein and Ureter Flow.

A few experiments on the following page, selected from a considerable number, will illustrate the phenomena.

B. The Cause of the Change in the Vein and Ureter Flow.

This was referred in the previous paper to a shrinkage or swelling of the renal cells, producing a widening or narrowing of the vascular and urinary channels. This explanation seems fully justified by some recently published investigations.

Heidenhain had already noticed, in sections, that the renal cells readily change their volume through osmosis. Von Sobieranski[2] has recently shown, by histologic examination, that the cells of the convoluted tubules are much

[1] Sollmann, Torald: This journal, 1903, ix, p. 459.

[2] v. Sobieranski, W.: Archiv für die gesammte Physiologie, 1903, xcii, p. 135.

shrunken, and the tubular lumen widened, if the kidneys (of rabbits) are excised during the diuresis produced by the intravenous injection of hyperisotonic salt solutions. After the injection of hypoisotonic solutions (0.65 per cent sodium chloride) the cells are swollen to an occlusion of the lumen. Hamburger[3] has also demonstrated the osmotic changes in

No. of experiment.	Solution changed from	to	Vein flow changes from	to	Ureter flow changes. from	to
	1. Changing from stronger solutions to weaker.					
65	5.0% NaCl	1.0%	180	120	112	28
	1.0% NaCl	¼%	120	<64	28	3½
	2. Changing from weaker solutions to stronger.					
65	1.0% NaCl	5.0%	100	180	1	112
	¼% NaCl	5.0%	<64	>200	3½	72
84	1.0% NaCl	2.0%	56	±200	12	50
	3. Even very small changes in concentration cause noticeable changes in the vein and ureter flow.					
66	1.0% NaCl	0.9%	19	15	1	⅔
	0.9% NaCl	1.0%	15	20	⅔	1⅓
	1.0% NaCl	1.1%	20	27	1⅓	1⅘
	1.1% NaCl	1.0%	27	23	1⅘	2
	4. The same changes are obtained in kidneys which have been excised a day before.					
63	1.0% NaCl	¼%	56	48	..	..
	¼% NaCl	1.0%	48	64	..	..
	1.0% NaCl	¼%	64	48	..	..
	5. It was shown in the previous paper[1] that *80 per cent alcohol* also acts as a hyperisotonic solution, as far as the vein flow is concerned. The ureter flow, however, is diminished through the coagulation of the protoplasm.					

[1] *Loc. cit.*, Experiment 37, page 426.

the volume of isolated renal cells by centrifugalization; the volume was about 1.3 times as great in a 0.6 per cent solution, as in a 1.5 per cent solution.

The fact that the kidney cells swell in hypoisotonic solutions, and shrink in hyperisotonic mixtures, was also proved by the changes of weight

[1] HAMBURGER, H. J.: Osmoticher Druck und Ionen Lehre, 1904, iii, p. 53.

which could be observed after laying sections of kidney, ½ mm. to 2 mm. thick, in the solutions. The sections were carefully dried with blotting-paper before weighing. The limit of error in this rough method did not usually exceed 0.017 gm. Conclusions are only justified when the observed difference exceeds this limit, or when all the specimens depart uniformly in the same direction, or if the difference increase with the time which the specimen remains in the solution. Two or three sections were observed in each case, and compared with an equal number of control specimens preserved in the 1 per cent sodium chloride solution.

The latter seems to be hypoisotonic to the kidney cells, for these gain in weight for over two and one-half hours. After eighteen hours, however, the weight remains constant. I shall only cite experiments made with the specimens which had reached this constant weight.

The following protocol will illustrate these statements:

Experiment 169.2.

Weight after 15 seconds in 1 per cent sodium chloride, 0.6237 gm.			
After	5 minutes, difference	= +	0.0243 gm.
"	30 " "	=	0.0521 "
"	1 hour "	=	0.0918 "
"	2½ " "	=	0.1423 "
"	18 " "	=	0.1943 "
Weight after	18 " "	=	0.8180 "
Difference in	18½ " "	= +	0.0110 "
"	19 " "	= −	0.0005 "
"	21½ " "	= +	0.0170 "

The differences after 18 hours are evidently errors, due to variations in drying.

When transferred to water, the sections increased very considerably in weight; in stronger salt solutions they lost weight:

PROTOCOLS.

Experiment 169.5.

Weight after 20 hours in 1 per cent sodium chloride		0.6695 gm.
(Increase) after lying in *water* for	½ hour	+ 0.1015 "
" " "	1 "	+ 0.1315 "
" " "	3½ "	+ 0.1155 "

Experiment 169.7.

Weight after 20 hours in 1 per cent sodium chloride		0.2660 gm.
(Loss) after lying in *2½ per cent sodium chloride* for	½ hour	− 0.0670 "
" " " "	1 "	− 0.0730 "
" " " "	3½ "	− 0.0570 "

The lessened resistance in the blood channels brought about by hyperisotonic salt solutions is also shown by several facts cited in the second paper; viz., the increased maximal vein pressure, and the lessened maximal ureter pressure when the vein is left open.

The increase of the ureter flow is partly due to the vascular dilatation, which tends to raise the filtration pressure; but a very considerable increase of filtration must also result from the lesser resistance to the passage of fluid through the dilated tubules. On account of this dilatation of the tubules, a larger vein pressure is required to obliterate them so as to stop the urine filtration.

C. Changes in the Volume of the Kidney Produced by Anisotonic Solutions.

The remarkably constant and very large influence which the concentration of the salt solution has upon the vein and ureter flow, would lead one to look for changes of similar magnitude in the volume of the kidney. This expectation is not fulfilled. Of all the agencies which we have found effective in influencing the ureter and vein flow in any appreciable degree, the concentration has the least and the most variable effect on the oncometer. Only water produces any great change. The reason for this is not far to seek: if the cells are shrunken by the solution, their place is taken by the fluid; if they are moderately distended, they simply displace so much fluid, the total volume of the kidney being but little altered.

Some slight changes, however, do occur, generally less than 1 cm. on the oncometer (equivalent to perhaps 10 cm. of water pressure in the injection tube). They begin very promptly, and are usually completed within ten minutes. The degree by which concentrations are changed seems to have only a minor influence on the degree of the oncometric changes; if the concentration varies by a very small amount (*e.g.*, by 10 per cent), there is no effect on the oncometer. Water, however, produced a large change.

The effects may be summarized as follows:

(a) **Change from lesser to greater concentrations.** – In practically all cases in which the oncometer was observed continuously, there was at first a sharp fall in the oncometer; in some cases this lasted but a few moments; in others, it lasted to five minutes. This fall is always followed by a compensatory rise toward or above normal, usually reaching its limit within eight or ten minutes, and then persisting. The preliminary fall of the oncometer indicates that the dilation involves, first, the vessels located rather toward the venous end of the circulation (the intertubular capillaries ?); the secondary rise in the oncometer shows that the dilation gradually extends towards the

arterial end (perhaps by dilation of the glomerular capillaries on account of the lessened resistance in the urinary tubules). The changes suggested may be represented diagrammatically as in Fig 1. In accordance with this explanation, it was observed (in Experiment 65) that the vein flow began to increase when the oncometer fell, whilst the ureter flow increased only when the secondary rise of the oncometer occurred.

(b) **Change from greater to lesser concentration.** — Hypoisotonic solutions lead to a progressive fall of the oncometer, which reaches

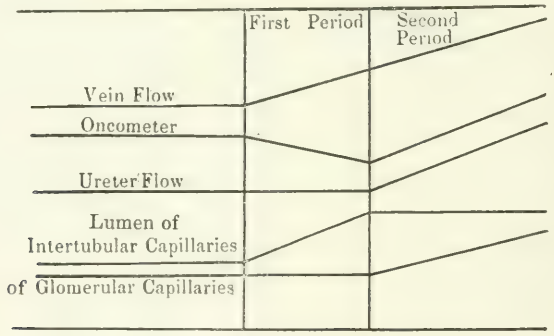


FIGURE 1. — Diagram of the effects of hyperistonic solutions.

its lowest level usually inside of five minutes, and does not return toward normal. (One experiment of seven showed a secondary rise; but since this was only observed once, and since the same kidney in a previous observation showed the typical persistent fall, I believe myself justified in attributing this exception to some accidental disturbance of the oncometer.)

The fall in the oncometer must be explained by a lesser amount of fluid in the kidney, caused by the partial or complete obliteration of the lumen of the vessels by the swollen cells. This obliteration cannot be confined to the venous end of the circulation (or the volume would be increased); but must involve the arterial end or the intermediate sections. It is probably localized mainly in the inter tubular capillaries.

(c) The fall in the oncometer is very much more pronounced if water is circulated, and it may then exceed 7 cm.

A few selected protocols may serve to illustrate the above statements and conclusions:

PROTOCOLS OF ONCOMETER OBSERVATIONS.

Time.	Change from	Oncometer.
	(*a*) Hyperisotonic Solutions.	
	Experiment 58 (second day).[1]	
10.45	1.0% to 0.5%	In 7 minutes fall of 0.5 cm. In 12 minutes return to original level.
	Experiment 63 (second day).	
9.30	¼% to 1.0%	In 5 minutes fall of 0.5 cm. In 6 minutes rise of 0.5 cm. above original level. In 8 minutes rise of 0.6 cm. above original level.
10.06	¼% to 1.0%	In 2 minutes fall of 2.4 cm. below original level. In 3 minutes fall of 0.6 cm. below original level. In 5 minutes fall of 0.8 cm. below original level.
	Experiment 65.	
10.30	¼% to 5.0%	In 2 minutes rise to 0.4 cm. above original level. In 3½ minutes fall of 0.4 cm. below; increased vein flow begins. In 4½ minutes rise to 0.2 cm. above original level. In 5 minutes rise to 0.4 cm. above; increased ureter flow begins. In 6 minutes rise to 0.5 cm. above. In 8 minutes stands at 0.5 cm. above.
	(*b*) Hypoisotonic Solutions.	
	Experiment 58 (second day).	
11.12	5.0% to 1.0%	At once, fall of 0.8 cm. below original level. In 5 minutes remains at 0.8 cm. below original level.
11.27	5.0% to ¼%	At once, fall of 0.8 cm. below original level. In 5 minutes fall of 1.0 cm. below original level. In 10 minutes fall of 1.8 cm. below original level.
	Experiment 65 (fresh kidney).	
10.15	5.0% to ¼%	In 2 minutes fall of 0.2 cm. below original level. In 4 minutes fall to 0.5 cm. below original level. In 16 minutes fall to 0.6 cm. below original level. In 19 minutes fall to 0.7 cm. below original level.

[1] *i. e.*, kidney excised on the previous day.

(*c*) Water.		
Experiment 99 (fresh kidney).		
Time.	Change from	Oncometer.
3 00	1.0% NaCl to water	In 5 minutes fall to 3.9 cm. below original level. In 8 minutes fall to 7.3 cm. below original level.

D. Application of the Results to the Conditions in the Living Body.

It could be objected to the application of these experiments to the living body, that the molecular concentration of the blood tends to return rapidly toward the normal, when anisotonic solutions are injected into the circulation. However, this return to the normal is not by any means completed so quickly; in the experiments of Galeotti [1] it was not completed in five hours. His curves (p. 211) illustrate how the diuresis varies inversely as the concentration of the blood. The observations of v. Sobieranski, quoted above, show still more strikingly that the results of the excised kidney may be extended to the conditions of the living body. A large part of the diuresis following the injection of hyperisotonic solutions may, therefore, be explained by mechanical principles.

It is scarcely necessary to add that all the phenomena of saline diuresis cannot be explained in this manner. For instance, as I pointed out in my previous paper on this subject,[2] glucose, urea, and iodide give a higher rate of diuresis than can be accounted for by their molecular concentration. On the other hand, sodium chloride or acetate gives a lesser diuresis.[3]

E. Conclusions.

The vein and ureter flow in the excised kidneys vary with the molecular concentration of the perfusion fluid. The effects are conspicuous and prompt. The results occur with even slight changes of

[1] Galeotti, G.: Archiv für Physiologie, 1902, p. 200.

[2] Sollmann, Torald: This journal, 1903, ix, p. 459.

[3] In the table on page 459 a misprint occurs in the diuretic factor for the acetate, which should be 4 instead of 1. I have also found, by direct experiment that the dissociation of this salt is almost complete, *i. e.*, near 2.

concentration, and may be elicited from kidneys which have lain a day, and also on the perfusion of alcohol.

The results are brought about by a shrinkage or swelling of the renal cells; the former (produced by concentrated solution) causing a lessened resistance in the vessels and tubules, hypoisotonic solutions having the reverse effects.

The changes in the volume of the kidney are relatively small (the variations in the volume of the cells being compensated by variations in the quantity of fluid). Definite changes do, however, occur. With hyperisotonic solutions there is at first a sharp fall, followed by a compensatory rise above the original. Hypoistonic solutions lead to a progressive fall of the oncometer. Water causes a sharp diminution in the volume. The results may be explained by the changes in the renal vessels.

The effect of the molecular concentration of the circulating fluid is in entire accord in the dead kidney and in the living animals.

The results obtained with the excised kidney emphasize the need of caution in interpreting oncometric data.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. IV. — SOLUTIONS OF NON-ELECTROLYTES.

By TORALD SOLLMANN.

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THE effects of solutions of non-electrolytes may be considered in this place, since the most conspicuous actions could be referred to osmosis.

Four substances were used, viz.: urea, alcohol, dextrose, and cane sugar. They were employed in solutions of the same freezing point as 1 per cent sodium chloride, and were alternated with the latter solution. (The solutions were tested by freezing point determinations. Their strength is approximately: Urea, 1.89 per cent; alcohol, 1.45 per cent; dextrose, 5.67 per cent; cane sugar, 10.88 per cent.)

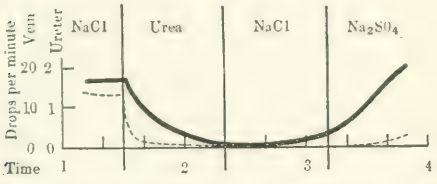


FIGURE 1. — Isotonic urea (Experiment 56). ——— denotes the vein flow; – – –, the ureter flow.

1. Urea. — The isosmotic urea solution was circulated in six experiments; the effects were the same in all, and consisted in a prompt and extensive fall of the vein and ureter flow and of the oncometer (Fig. 1). The results are also obtained in kidneys which were excised on the preceding day.

The effect on the vein flow is removed completely by sodium chloride, sodium sulphate, or magnesium chloride; the effect on the ureter flow persists after sodium chloride or sulphate, but is promptly removed by magnesium chloride.

The same phenomena (in a lesser degree) occur when the solution of urea is mixed with solution of sodium chloride (1 part, urea; 3 parts, isotonic sodium chloride).

The phenomena do not occur if urea in substance is added to the sodium chloride solution (as much urea as would make a half isotonic

solution if added to water); if anything, there is a trifling increase of vein and ureter flow.

The action of the urea on the vein and ureter flow corresponds with the effects of very hypoisotonic solutions. In other words, the urea must penetrate the renal cells in the same way as it penetrates the blood corpuscles. The absence of the specific effects of urea, when it is added in substance to isotonic salt solution, also proves clearly that its actions are in the main based on osmotic changes; the slight increase in the latter experiment shows that the permeability of the renal cells to urea is not absolute.

2. **Alcohol.** — The effects of an isotonic solution are shown in Experiment 94:

Exp.	Perfusion with	Time.	Vein flow.	Ureter flow.	Oncometer.
					cm.
94	NaCl	4.00 P. M.	200	36	22.6
		4.10 P. M.	200	34	22.9
		4.20 P. M.	200	40	22.9
	Alcohol . . .	4.26 P. M.	..	..	..
		4.27 P. M.	24	8	20.3
		4.30 P. M.	6	12	18.4
	NaCl	4.31 P. M.	..	..	..
		4.38 P. M.	200	36	22.5
		4.44 P. M.	200	38	22.6

They are so similar to those of urea, that the curves need not be reproduced. The explanation of its action must be the same as with urea, and the experiments made with the latter were not repeated. The permeability is also incomplete with alcohol, so that strong solutions increase the vein flow.[1]

The swelling of the kidney cells in isotonic solutions was also shown by the method described in the preceding paper (page 280).

Experiment 169.21.

Weight of the section after 20 hours in 1 per cent sodium chloride	0.776 gm.
Increase of weight after lying in isotonic alcohol for ½ hour	+0.123 "
" " " " 1 "	+0.169 "
" " " " 4 "	+0.137 "

[1] Sollmann, T.: This journal, 1903, ix, p. 462, Experiment 37.

3. **Dextrose.**—The effect of this solution, when compared with sodium chloride, was small and inconstant. There was generally a trifling diminution in the vein flow and increase in the ureter flow.

4. **Cane sugar.** — Six experiments were made. The effects are never very large, are always removed promptly by sodium chloride, and are probably pure viscidity phenomena.

As a rule, the vein flow is first increased (from the increased injection pressure resulting from the high specific gravity of the solution). The vein flow is then slightly diminished (as the solution reaches the capillaries and increases the friction by its viscidity). The ureter flow undergoes corresponding changes (in the same direction as the vein flow) from the same causes.

5. **The effect of these substances in the intact body.** — I have shown in a previous paper [1] that the diuretic effect of dextrose, urea, and alcohol is considerably greater than that of equimolecular sodium chloride. Similar results were found by Haake and Spiro [2] in regard to cane sugar and glucose. The present experiment shows that this cannot be reproduced in the excised kidneys. So that the specific diuretic effect in animals must either be connected with the living condition of the kidneys, or else the conditions must be different when these substances are added to the complex mixture of ingredients which form the blood plasma, than when they are used alone.

The very injurious effect of urea on the renal cells may cause surprise. It must be remembered, however, that the salts of the urine would prevent these effects, which are only produced in pure watery solutions.

Conclusions. — Solutions of urea and alcohol, of the same freezing point as 1 per cent sodium chloride, act like strongly hypoisotonic solutions, penetrating freely into the renal cells. This permeability is not, however, absolute.

Isotonic solutions of dextrose and cane sugar cause only small and somewhat variable changes, which can, for the most part, be ascribed to viscidity.

[1] Sollmann, T.: This journal, 1903, ix, p. 459.

[2] Haake, B., and Spiro, K.: Beiträge zur chemischen Physiologie und Pathologie, 1902, ii, p. 149.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. V.—THE EFFECT OF VISCIDITY.

By TORALD SOLLMANN.

(With the Collaboration of R. A. Hatcher.)

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Egg albumen. — A solution of egg white in 1 per cent sodium chloride, containing 2.445 per cent of proteid, was employed in Experiments 88 and 89. The results are shown in Fig. 1. They consist in a diminution of the vein and ureter flow, and a decrease of the volume of the kidney. The effects occur promptly, and are profound. They are but slowly and imperfectly removed when the perfusion with saline solution is resumed, presumably because the albumen is very slowly washed out, owing to its adhesive property.

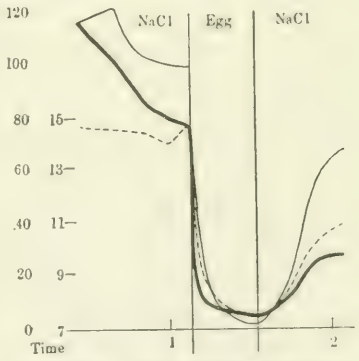


Figure 1. — Egg albumen (Experiment 89). ▬▬, vein flow; ———, ureter flow; - - - oncometer. (Flow = drops per minute; oncometer = cm.)

The effects are readily explained by the increased friction. The fall in the oncometer shows that this makes itself felt mainly on the arterial side of the circulation.

Precisely similar results were obtained when the albumen was added to 2 per cent sodium chloride (Experiment 126.5). Here, also, the recovery was very imperfect.

Acacia. — A 1 per cent solution of gum acacia in 1 per cent sodium chloride was employed in Experiments 84 and 85, but produced no constant effect; a 5 per cent solution, however, tried in the same experiments, agreed with the egg solution in causing a prompt and persistent diminution of vein and ureter flow and oncometer. The effect was not as great as with the egg. Subsequent perfusion with sodium chloride caused the recovery of the ureter flow, but the oncometer and vein flow did not recover.

A part of the effect of the acacia must be referred to its mineral constituents, for the ash of the specimen used in these experiments had a similar action (Experiment 89). This is probably due to the calcium, which is present in considerable amount; for, as I shall show in a later paper, calcium chloride also produces analogous effects.

I have already mentioned, in the fourth paper, that the effect of *cane sugar* solution may be referred to its viscidity, whilst glucose solutions show very little of this action.

Conclusions. — Viscidity causes a diminution in the vein and ureter flow, and in the volume of the kidney.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. VI.—THE ACTION OF BLOOD ON THE KIDNEY.

By TORALD SOLLMANN.

[*From the Pharmacological Laboratory of Western Reserve University, Cleveland, Ohio.*]

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A. Introductory.

THE comparatively great viscidity of blood[1] led me to expect that its perfusion would produce effects analogous to those described in the last paper. This proved to be the case when the kidneys were perfused a day after excision, or later.[2]

In perfusions undertaken within eight hours after excision, the ureter flow and the oncometer also agreed with the viscidity effects, *i. e.*, they were diminished. The vein flow, however, generally showed the opposite results, *i. e.*, it was increased, being often more than twice as great as with salt solution. This very surprising result led me to undertake an extensive investigation into its cause and mechanism. It was found that there are two factors at work, which influence the vein flow in opposite directions, viz. the retarding factor of viscidity, and an accelerating factor, furnished by a dilation of the renal vessels.

The dilation is active, and exceeds greatly the passive post-mortem dilations of these vessels. It probably involves the efferent arterioles. It is exerted by an organic, probably proteid, constituent of the

[1] Hürthle, K.: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 415; Opitz, R. Burton: *Ibid.*, p. 447; This journal, 1902, vii, p. 243. According to Opitz, the outflow of serum through a capillary tube is 2½ to 4½ times as great as that of blood.

[2] Dog's kidneys and defibrinated dog's blood were employed in all the experiments.

serum, the activity of different serums being variable. The dilator-response disappears with the vitality of the renal vessels, and is indeed variable for different kidneys soon after excision.

On account of these variations in the kidneys, and in the relative predominance of the viscidity and dilator effects of different bloods, I made a rather lengthy series of experiments, before I felt justified in drawing conclusions. The series comprises a total number of 238 perfusions with blood or serum, made on more than 30 kidneys. The results of every experiment were plotted as curves, from which the conclusions are deduced. Only a few of these experiments are quoted for illustrating; with very few exceptions, every deduction is based on at least five experiments.

I am not yet prepared to say whether blood has a similar dilator-action on the vessels of other organs. The experiments in this direction are not yet completed. So far the results are rather negative.

B. Perfusion of Dead Kidneys with Blood.

These illustrate the pure effects of viscidity, as described in the preceding paper. Fourteen kidneys were used, two days after excision. Without exception, the substitution of blood for salt solution caused a prompt decrease of oncometer and vein flow. (The ureter flow could not be observed, as it is extremely slow in kidneys which are two days old, even when salt solution is perfused.) The vein flow is practically abolished, even when the viscidity has been greatly reduced by laking and dilution. Serum, since it is much less viscid, has much less effect (see Fig. 1). The same phenomena are produced when the kidney vessels are dilated by 2 per cent sodium chloride solution.

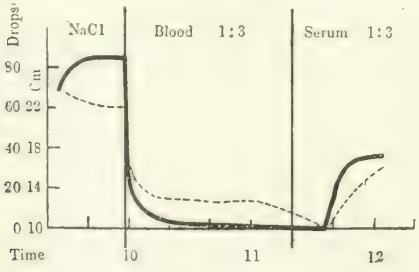


Figure 1. — Effect of blood and serum on the dead kidney (Experiment 104). (General explanation of the figures: ▬▬ = vein flow; ——— = ureter flow; - - - = oncometer. Unless otherwise stated, the vein and ureter flow are given as drops per minute; the oncometer in centimetres on an arbitrary scale. "Blood 1:3" signifies one part of defibrinated blood and three parts of 1 per cent sodium chloride solution. "NaCl" signifies 1 per cent sodium chloride solution.) The vertical lines limit the beginning and end of each perfusion.

C. General Phenomena of the Perfusion of Recently Excised Kidney with Blood.

The typical phenomena are shown in Fig. 2. From this it may be seen that the vein flow is suddenly increased, the maximum being usually attained within five minutes. There is then a sharp return of the vein curve toward a constant, which is generally reached within fifteen or twenty minutes. This constant is usually about the level of the original vein flow, but may be either above or below.

The *oncometer* shows a sharp and severe fall from the start. This is quite independent of any changes in the vein flow. (The slight irregularity in the figure is unusual, and probably accidental.)

The *ureter flow* also shows a sharp and conspicuous decrease, being always reduced to a very few drops. In most cases this is followed by a slight improvement when the vein flow diminishes, as shown in the figure.

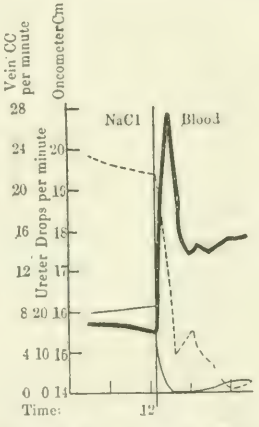


Figure 2. — Typical effect of blood, living kidney (Experiment 89).

As I have indicated in the introduction, these typical changes of the vein flow are not obtained in every experiment, because there are a number of interfering factors. The discrepancies are easily explained when the mechanism of the action of blood is understood. I shall, therefore, take up this latter subject first.

The effects on the vein flow. — The increase of the vein flow admits of but one explanation, viz., a dilation of the blood vessels, of such a degree as to more than counterbalance the viscidity (which would practically abolish the vein flow, as in the dead kidney). This dilation, however, is peculiar in that it is accompanied by a lessened volume of the kidney. Consequently, the dilation must occur at a limited and constricted portion of the vascular channel (for if a tract of large area were dilated, this would by itself increase the volume of the kidney). Furthermore, the dilation must occur at a point where it will favor the flow of liquid *from* the kidneys rather than the flow *into* the kidney. It is evident that the situation which complies best with these requirements corresponds to the efferent arterioles of the

glomeruli. In this respect, the dilator effect of blood differs markedly from that of most (if not all) other dilator agencies which act mainly on the afferent arterioles, and which consequently increase the volume of the kidney. However, there can be little doubt that blood also has some dilator effect on the afferent arterioles. For this speaks the increase of the maximal vein pressure (see second paper of this series). It is also shown by the fact that the diminution of the oncometer *may be* relatively quite small, especially when the viscidity is reduced (as with serum and laked blood) (Fig. 3). (This also shows that a considerable part of the oncometer decrease is due to the viscidity.)

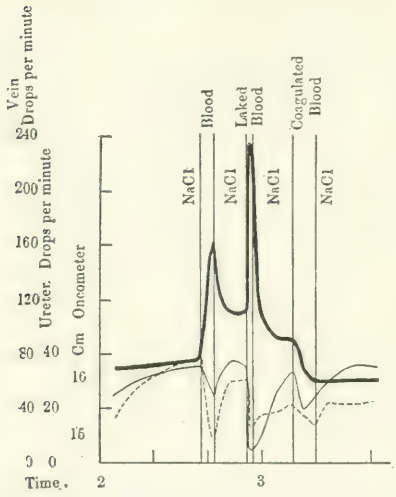


FIGURE 3. — Effect of blood, laked blood, and coagulated blood (Experiment 162). All the bloods are diluted with three volumes of salt solution.

The rapidity with which the maximum of vein flow is reached (usually within five minutes) shows that the dilator response is very prompt.

The next phenomenon to require explanation, is the sharp *secondary fall of the vein flow* (see Fig. 2). This might be explained by a wearing off of the dilator effect; this is very unlikely, considering the rapidity of the phenomenon, and the fact that the vein flow generally remains constant above that of saline solutions. A sufficient explanation is furnished by the observation (which will be discussed later) that moderate dilution of the blood has less effect on the dilation than on the viscidity. When the perfusion of the kidney with blood is started, the first portions of blood are diluted with salt solution, the viscidity is less, and the flow consequently greater. In proportion as the blood in the vessels becomes less dilute and more viscid the vein flow diminishes.

If this explanation is correct, we should expect to find the secondary fall most constant and most pronounced, the less the original dilution of the perfused blood. This is indeed the case.

For instance, with undiluted blood, or blood diluted with an equal vol-

ume of salt solution, the secondary fall occurred in every one of nine experiments; with greater dilutions (1 : 3 to 1 : 6) it occurred only in $\frac{9}{15} = 60$ per cent of the cases.

When the primary increase of vein flow was above 100 per cent, the secondary fall occurred in every one of ten experiments; with the primary increase between 10 and 100 per cent, the secondary fall occurred only in $\frac{8}{14} = 51$ per cent of the cases.

Effect on the oncometer. — The oncometer (see Fig. 2) shows a steady fall, — at first abrupt, then more and more gradual. This fall appears to be quite independent of the vein flow, for it occurs when the vein flow is increased (*e. g.*, in Experiments 98 and 121), or when it is diminished (Experiments 108, 116); and the smoothness of the oncometer curve generally gives no indication of the sharp nose in the vein flow curve. The oncometric change is to be explained mainly as an effect of viscidity, and is generally proportional to the latter. However, in view of the increased vein flow, the viscidity can scarcely be alone responsible for the fall in the oncometer, and it is necessary to assume a dilation of the efferent vessels which would also cause a fall in the oncometer, as I have previously explained.

The oncometer shows no recovery during the perfusion of blood, as a rule. In some rare cases, small oscillations are seen, which are probably accidental. They are too inconstant and too slight to have any significance.

The effect on the ureter flow. — In the second paper of this series, I have shown that the perfusion of blood causes a diminution of the ureter pressure. The ureter flow is also diminished in every case and often temporarily abolished. This result occurs whether the previous diuresis was poor or good. The effect is generally proportional to the viscidity, and is to be attributed mainly to the great resistance which viscid solutions offer to filtration. However, the predominance of the dilation of the efferent vessels, which I have assumed, would also lower the filtration pressure, and therefore act in the same direction.

The ureter flow usually reaches its minimum within five, or at most ten, minutes, and then remains constant, or shows a very slight recovery, as in Fig. 2.

It would not be profitable to attempt an explanation of this slight recovery. It apparently has some connection with the secondary fall of the vein flow. It was uniformly absent in fourteen experiments, in which the

vein flow did not show the initial increase. It occurred in twelve out of nineteen experiments in which the initial increase of vein flow was observed; and in these cases it coincided in time with the secondary fall of the vein flow, as in Figs. 2 and 8.

D. Effect of Various Conditions on the Dilator Phenomenon.

The relative frequency of the typical results may be judged from the following table, which comprises only uninjured kidneys (excluding duplicates, and ineffective dilutions):

In a material of twenty-eight kidneys, the perfusion of blood (generally diluted) causes the vein flow to

Increase in 21 (75 per cent).
Decrease in 5 (18 per cent).

Two kidneys showed both increase and decrease, according to conditions.

In a material of forty-three perfusions (on the above kidneys) the blood caused the vein flow to

Increase in 34 (79 per cent).
Decrease in 8 (18 per cent).

In one case there was no change.

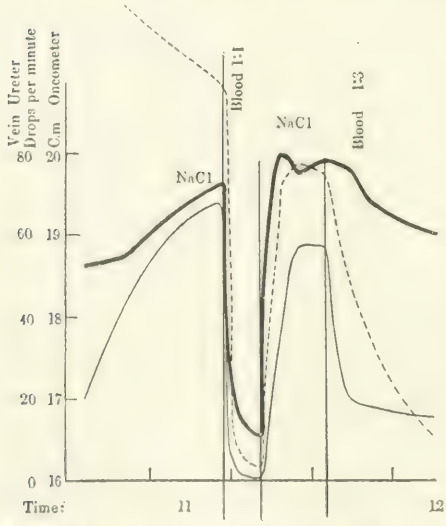


FIGURE 4. -- Perfusion of blood, showing decrease of vein flow (Experiment 108).

It will be seen that the typical increase of vein flow occurs in $\frac{3}{4}$ to $\frac{4}{5}$ of the cases. The others show a decrease of vein flow (Fig. 4), or no appreciable effect. However, a very considerable dilation must have occurred even in these cases, for the slowing is never as great as the viscidity demands (*i.e.*, as occurs in dead kidneys) (compare Figs. 1 and 4).

To show the quantitative differences, I have compiled the results on the vein flow into groups, as follows:

Group A.	Increase above 100 per cent.	12 perfusions (28 per cent).
Group B.	Increase of 25 per cent to 100 per cent.	11 perfusions (26 per cent).
Group C.	Increase of 10 per cent to 25 per cent.	8 perfusions (18 per cent).
Group D.	Increase just noticeable.	3 perfusions (7 per cent).
Group E.	No effect.	1 perfusion (2.5 per cent).
Group F.	Decrease of 10 per cent to 25 per cent.	3 perfusions (7 per cent).
Group G.	Decrease of 25 per cent to 50 per cent.	4 perfusions (9 per cent).
Group H.	Decrease below 50 per cent.	1 perfusion (2.5 per cent).

The factors which appear to be of principal importance in producing these quantitative differences are the following :

1. The period of the perfusion. — Arranging the experiments according to the period after the excision when the perfusions were made, it is found that the proportion of cases which give an increase of vein flow is :

24 out of 29 (83 per cent), in perfusions made within an hour after excision.
8 out of 10 (80 per cent), in perfusions made between one and three hours.
6 out of 10 (60 per cent), in perfusions made between three and seven hours.
0 out of 14 (0 per cent), in perfusions made after forty-eight hours.

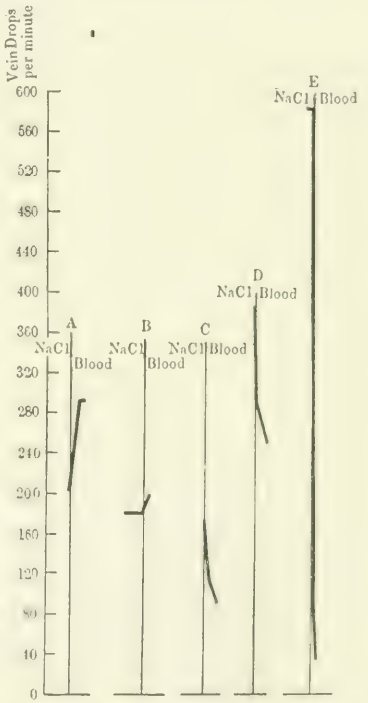


FIGURE 5. — Effect of time of perfusion (Experiment 137). The kidney is perfused with defibrinated blood diluted with two volumes of 1 per cent salt solution ; (*A*) $1\frac{3}{4}$ hours after excision ; (*B*) $3\frac{3}{4}$ hours ; (*C*) 24 hours ; (*D*) 29 hours ; (*E*) 50 hours.

The difference, up to three hours, is not very great, but it can be seen quite plainly that the proportion of experiments showing an increase of vein flow diminishes with the time which elapses after the excision. The study of individual experiments in which the perfusions were repeated at intervals, shows still more strikingly that the dilator reaction becomes progressively smaller (Fig. 5). The decrease is at first abrupt, and becomes more and more gradual the longer the time elapsing after excision. The time when the reaction seemed to be completely abolished coincided with the time when the reducing power for hæmoglobin disappeared. It was

absent in every case forty-eight hours after excision. Eighteen hours after death, it was sometimes present and sometimes absent.

A conspicuous manifestation of the same phenomenon is seen in the relative frequency of large dilator effects in the different periods of perfusion:

Of twelve cases in which the vein flow was increased more than 100 per cent, eleven were made within the first hour; or to put the same matter in another way, of twenty-nine perfusions made within the first hour, eleven, or 38 per cent, gave an increase above 100 per cent; whilst of twenty perfusions made at later periods, but one (5 per cent) exceeded 100 per cent.

Explanatory. To explain the greater efficiency in the earlier period, it might be assumed that there is at this time a tonic contracture of the vessels, and that the blood acts merely by relieving this tonus. It will be recalled, however, that the curve for perfusions under constant conditions (first paper) does not show any diminution of tone during the first five hours of perfusion; on the contrary, the vein flow is decreased. After twenty-four hours there is indeed in every instance, a considerable increase of vein flow (as in Fig. 5, *D* and *E*); *i. e.*, a marked loss of vascular tone; but it cannot be argued that the dilator effect of the blood is due to the removal of this tone, else the absolute vein flow during the perfusion of blood should be the same, approximately, in all periods, which is not the case.

There seems to be but one explanation for the phenomenon, viz., that the dilation is an active process, which requires the life of the vasomotor structures of the kidney, and that the response of this peripheral dilator mechanism lessens with any injury, and thus with the time elapsing after excision.

2. Injuries. — The dilator reaction is considerably diminished by perfusing, for one-half to one hour, with sodium fluoride (0.3 per cent added to 1 per cent sodium chloride), or with 2 per cent sodium chloride. After these perfusions, blood causes a slowing of the vein flow, whereas before the injury, the vein flow was increased by the blood. The flow is much better, however, than in dead kidneys, so that the dilator reaction is not completely destroyed. The injury occurred in every experiment in which the strong salt solution was perfused. The effects of the fluoride were less constant, as they occurred in only half the experiments.

3. Differences in kidneys and in blood. — When the same blood is perfused under similar conditions through the two kidneys of the same dog, the effects are very similar. This uniformity does not

obtain in experiments on different animals. The variability could be be referred to differences in the reacting power of different kidneys, or to differences in the dilating power and in the viscidity of different bloods. It seems to me that all three factors are concerned; but it is difficult, from the nature of the experimental material, to prove this opinion. The differences in the time at which the dilator reaction disappears is strongly suggestive of differences in the kidneys. There is also some direct evidence of differences in the dilator power of different bloods.

E. The Effect of Diluting the Blood.

Dilution tends to affect the vein flow in two opposite directions: By reducing the dilator action, it tends to lessen the vein flow; by reducing the viscidity, it tends to increase the flow. The vein flow, therefore, may be either decreased or increased by dilution, according to which of the two actions predominates. The ureter flow and oncometer vary more uniformly, since they are influenced almost exclusively by the viscidity. They are therefore increased proportional to the dilution.

The effect of dilution may be studied by comparing the proportion of experiments in the entire series which show an increase of vein flow, arranging the perfusions according to the dilution of the blood. The proportion is found to be greatest when the blood has been diluted with two to five parts of salt solution. The proportion is distinctly less when undiluted blood is used, or blood which has been diluted with only one part of salt solution. The proportion is least, when the blood is diluted with seven or more parts of salt solution.

It appears, therefore, that moderate dilutions are more favorable to increased vein flow than either very slight or very great dilutions. This conclusion is fully confirmed by the data obtained by perfusing different dilutions of blood through a single kidney. The optimum dilution varies somewhat in different experiments: in those in which the dilator reaction is small, or the viscidity great, the best flow is obtained when the blood is more diluted.

These results can be understood by assuming that the viscidity is reduced in direct proportion to the dilution; whereas a moderate dilution reduces the dilator reaction proportionally less than great dilution, *i. e.*, according to a curve, somewhat as in Fig. 6. In other words, a blood diluted with three volumes of salt solution dilates the vessels almost as powerfully as undiluted blood, whilst

the viscidity is decreased to one-third. This fact has a practical importance, because the dilutions which occur in the body are always comprised within these limits. Accordingly, the renal effects of hydræmia correspond to those of a reduction of viscidity.

To reproduce the conditions of hydræmia more accurately, a number of experiments were made in which undiluted blood was alternated with various moderate dilutions. The results are illustrated by

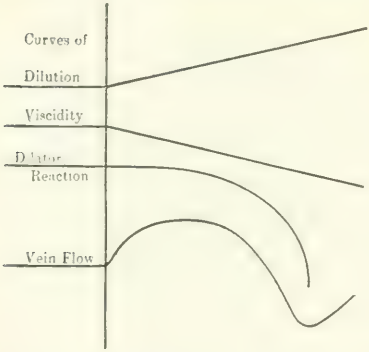


FIGURE 6. — Diagram to explain the effect of dilution on the vein flow.

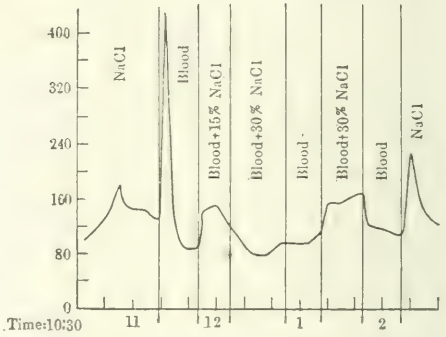


FIGURE 7. — The effects of diluting the blood (Experiment 123) on the vein flow (drops per minute).

Fig. 7. This shows that the vein flow is proportional to the dilution. The same holds true of the ureter flow and oncometer.

The phenomena agree exactly with those produced in living animals as the result of saline injections. Here also there is observed an increase of the vein flow, ureter flow, and oncometer. This is currently supposed to require a vital explanation, since the general blood pressure is not increased. In my experiments, the injection pressure is of course also kept constant. There can be no doubt that all the phenomena can be explained very simply by the lessened viscidity, *i. e.*, without assuming any vital vasodilation.

The effect of diminishing the viscidity is also seen by the perfusion of laked blood or serum, which will be described presently. These also cause a greater increase of vein flow, and a lesser decrease of the oncometer and ureter flow, as compared with equal dilutions of defibrinated blood.

The changes produced by substituting sodium chloride solution for blood. — In kidneys in which the viscidity effect predominates, the resumption of the saline perfusions lead to the changes which have just

been described, viz., an increase of the vein and ureter flow and oncometer. This is seen most typically in dead kidneys, and also in those experiments on recently excised kidneys in which the dilator effect was small (Fig. 4). Where the dilator effect of the blood is very pronounced, the effect of the salt solution on the vein flow is the opposite, *i.e.*, it is diminished (Fig. 3). This is easily explained by the disappearance of the dilation.

In the majority of experiments, however, the vein flow shows two phases: a primary increase, completed within five to ten minutes, and a secondary fall, to about the original level (see Figs. 7 and 8). The changes in the vein flow therefore bear a superficial resemblance to those produced by blood. The effects on the ureter flow and oncometer are, however, diametrically opposed, for these are always decreased by blood and increased by saline perfusion.

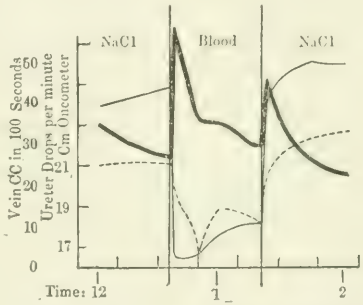


FIGURE 8. — Typical effects of sodium chloride following blood (Experiment 95).

The curve of the saline perfusion is easily understood, if the effect of dilution is recalled (Fig. 6). The primary rise in the vein flow is evidently due to the dilution of the blood within the kidney to the optimum concentration; or, in other words, the first portions of saline solution serve to lessen the viscidity, without interfering with the dilator effects, and the vein flow consequently increases. As the proportion of salt solution rises, the dilator effect disappears, and the vein flow falls toward normal. The effect on the oncometer and ureter flow being more strictly viscidity phenomena, these must show a steady rise, proportional to the gradual dilution, and independent of the vein flow.

F. The Nature of the Dilator Substance of the Blood.

1. Is oxygen concerned in the dilator phenomenon? — To answer this question, I compared oxygenated blood with blood which had been saturated with carbon monoxide until it was no longer reduced by a large excess of ammonium sulphide. The results show that the dilator effect is practically just as large in either case.

The oxygen dissolved in the serum could have no effect, since this is nearly alike in blood and in salt solution.

I have also compared oxygenated blood with blood which had been rendered very venous by passing it eight times through the kidney. This also showed no difference whatever.

It may therefore be concluded that oxygen is not concerned in the dilator phenomenon.

(Lastly I tried to paralyze the internal respiration by hydrocyanic acid, but the method proved unavailable, since I found that this poison has a very conspicuous specific dilator action on the renal vessels.)

2. **Are the corpuscles concerned in the phenomenon?** — Ordinary (diluted) blood was compared with blood which had been completely *laked* by heating to 60°–63° C. for fifteen to thirty minutes and filtering. It was found that the laked blood increased the vein flow even rather more effectively than the entire blood (Fig. 3).

This result made it necessary to determine whether serum possesses any dilator action. The sera were obtained by bleeding large dogs into 1 per cent salt solution, allowing the mixture to clot, and comparing the resulting serum with equivalent dilutions of defibrinated blood of the same animals.

The results showed that the increase of vein flow was considerably larger than with either defibrinated or laked blood. The ureter flow and oncometer were also much less diminished than by the blood.

It may be concluded that the corpuscles are not the essential cause of the dilator phenomenon; but that they rather hinder the increase of the vein flow by their viscidity (as illustrated on the dead kidney, Fig. 1).

It cannot be said, however, that the corpuscles are devoid of dilator action. Indeed, it would seem that the difference in the effects of serum and corpuscles is not as great as one would expect from their relative viscidity. I have tried to test this point by comparing the effect of blood with that of washed corpuscles, suspended in saline solution. The difference was not great; but the corpuscles were perhaps not sufficiently washed, on account of the practical difficulties, to permit any conclusions.

3. **Which constituent of serum is responsible for the dilator action?** — The dilator effect is not due to the mineral constituents of the serum. This was shown in two ways: (a) The mixture of salts contained in Locke's solution (*i.e.*, omitting the dextrose) is quite devoid of the dilator effect (although it causes a slight increase of ureter flow). Sodium bicarbonate, added to salt solution in the strength of Locke's

fluid, is also ineffective. (b) Blood coagulated by heating in the steam bath, and filtered, causes no dilation, but on the contrary a perceptible diminution of the vein and ureter flow and oncometer, due to the slightly lower molecular concentration (see Fig. 3). (The blood was heated at its natural reaction. Some turbidity was produced by adding acetic acid to a sample of the clear boiling liquid; this amounted (in Experiment 164) to 0.106 per cent of proteid in the filtrate from a blood which had been diluted with three volumes of salt-solution.)

It may therefore be concluded that the dilator principle is of organic nature; that it is not impaired by heating for half an hour at 60° to 63° C., but that it is either destroyed or precipitated between this temperature and 100° C.; or that it is enclosed by the coagulated proteid. This renders it probable that it is itself a coagulable proteid; but this is as far as I have carried its investigation.[1]

A 5 per cent solution of commercial dried serum albumin seemed quite devoid of the dilator action, showing pure viscidity effects.

G. Conclusions.

The general conclusions which may be deduced from these experiments are formulated in the introduction, and need not be repeated.

They are important in that they show:

(1) The existence of a dilator effect of blood.

(2) That the phenomena of saline diuresis (exclusive of the composition of the urine) can be reproduced in excised kidneys, and that the phenomena can be explained by the diminished viscidity of the blood, accompanying moderate hydræmia; and that it is therefore not necessary to invoke any vital mechanism to explain the increased volume of the kidney, the increased ureter flow, and the increased ureter pressure.

This conclusion does not, however, cover the changes in the composition of the urine, with which the present experiments do not deal.

[1] Laked blood which has stood a month at room temperature is apparently as active as fresh blood. It has a very putrid odor, but it still gives a solid coagulum on heating.

ON ERRORS OF ECCENTRICITY IN THE HUMAN EYE.

By CHARLES S. HASTINGS.

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ON pages 108 and 109 of the second edition of Helmholtz's great work on Physiological Optics is found a discussion of certain observations on the human eye from which he concluded that the lens of the eye is not, in general, truly centred. The method of investigation, the results of observation (given to the nearest tenth of a degree), and the conclusion were as follows:

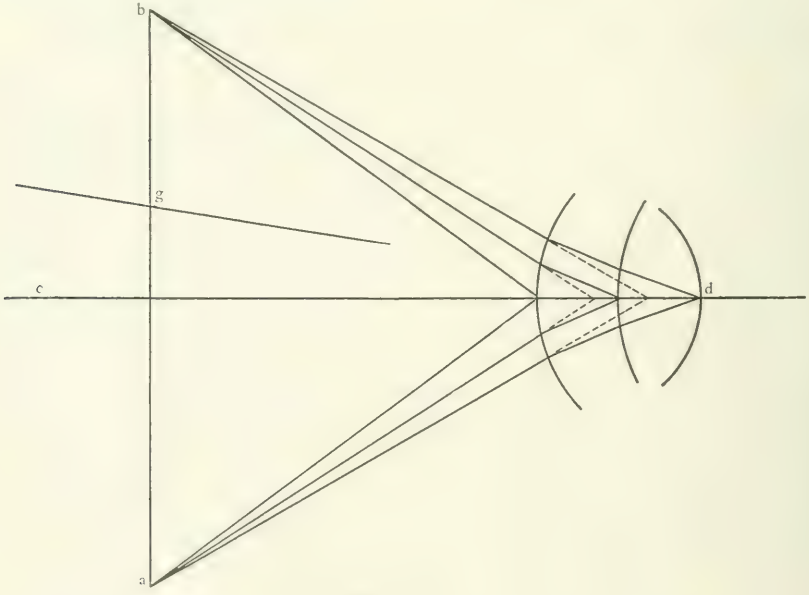


FIGURE 1.

Let *a b* be a horizontal scale at the ends of which are suitable openings for the observer's eye and the source of light. The eye to be investigated is placed at a point in the line *c d* which stands at right angles to the scale at its mid-point *c*. The scale has a movable object, *g*, upon which the axis of vision is to be directed; then *g* is

to be shifted until the observer finds that the reflected image from the front surface of the lens falls just between those formed by reflection from the cornea and from the back surface of the lens. After this condition is established the positions of the light and of the observing eye are exchanged, and the observer notes whether, the place of the fixations point remaining unchanged, the relative positions of the three images by reflection are retained. If the observed eye is perfectly centred, it must be possible (according to Helmholtz) to find a position for *g* which will meet this condition. Experiment showed that this could not be done, but that the horizontal projection of the line *d g*, always lying on the nasal side of *c d*, formed the following angles with the latter line:

Observer.	Eye.	Source lies with respect to *c d*	
		On temporal side.	On nasal side.
Helmholtz . . .	O. H.	3.8°	5.0°
	B. P.	5.1°	8.2°
	J. H.	5.7°	7.7°
Knapp	J. S.	4.8°	6.8°
	H. S.	3.7°	6.4°
	F. S.	4.6°	7.9°
	H. Sch.	6.1°	5.7°

"From this it follows that the human eye *is not accurately centred*."

There is nothing which should surprise us in the conclusion which is, after all, simply that the eye is not a perfectly constructed optical instrument; nevertheless, since all other optical defects had already been discovered in the eye, it may be regarded as interesting to demonstrate the existence of this kind also. Indeed, it does not seem unlikely that the interest of Helmholtz was restricted to this point. But if it were apparent that the error in question is systematic, it assumes a far wider and more philosophical importance; just as, for example, the fact that all human hands differ among themselves is of interest but of no great moment, but the fact that all left hands differ from all right hands in a definite and systematic way is of the greatest importance both physiologically and philo-

sophically. A glance at the table suggests that there is such a systematic difference, since the figures of the first column are smaller than the corresponding number in the second in every case excepting the last, where the difference is so small that it might be regarded as within the limit of error of observation. A critical inspection, however, does not yield a positive conclusion. It will be remarked, first, that Helmholtz does not reduce his conclusions to a quantitative form; second, that the differences might be supposed to indicate an error in collimation (or inclination of the axis of the lens to the axis of the eye) just as probably as an error of centring; and, third, that it is impossible to conclude from the observations, if an error of centring be assumed, even as to the direction of displacement. These considerations seem to make it worth while to discuss the observations in a more rigid manner than Helmholtz deemed necessary.

To this end I have calculated the positions of the three virtual images which are involved as formed by a schematic eye (Helmholtz, p. 140), namely, that formed by reflection at the surface of the cornea; that formed by refraction at the cornea, reflection at the anterior surface of the lens and final refraction at the cornea; and, finally, that formed by successive refractions at the cornea and front surface of the lens, reflection at the posterior surface of the lens and then refractions, in inverse order, at the two surfaces passed in emerging. The source was arbitrarily chosen as a point in the axis of the eye 100 centimetres in front of the vertex of the cornea. These images may be indicated by the symbols I_1, I_2, I_3, their distances from the vertex of the cornea being, respectively, 0.3899, 1.051, and 0.3942. The same calculations yield the ratios of the sizes of the images; they are $I_1 : I_2 : I_3 = 1 : 1.908 : -0.7297$. These results are represented graphically in Fig. 2.

Imagine the source displaced from the axis by the angle ϕ_1, as in the figure, then the ordinate of I_1 would become $0.3930\phi_1$, the number being the distance of the image I_1 from the geometrical centre of the cornea, while the other images would be displaced by amounts proportional to the ratios of magnification given above. We should thus have the following values for the ordinates of the three images:

$$y_1 = \quad 0.3930\ \phi_1,$$
$$y_2 = \quad 0.7492\ \phi_1,$$
$$y_3 = -0.2867\ \phi_1.$$

To an observing eye at a great distance in the direction ϕ_2, the projection of a point midway between the images I_1 and I_3 on a plane perpendicular to the axis and close to both of them would be at $\frac{1}{2}(y_1+y_3) = 0.0532\phi_1$. Similarly, the projection of I_2 on the same plane would be at $y_2 + 0.6590\phi_2$, that is, at $0.7498\phi_1 + 0.6590\phi_2$.

The conditions of Helmholtz's observations were, first, that the angular distance between the source and the observing eye was constant, that is, that $\phi_1 - \phi_2 = \pm 2\psi$, the upper sign holding for the first position of the source and observer and the lower for the interchanged position.

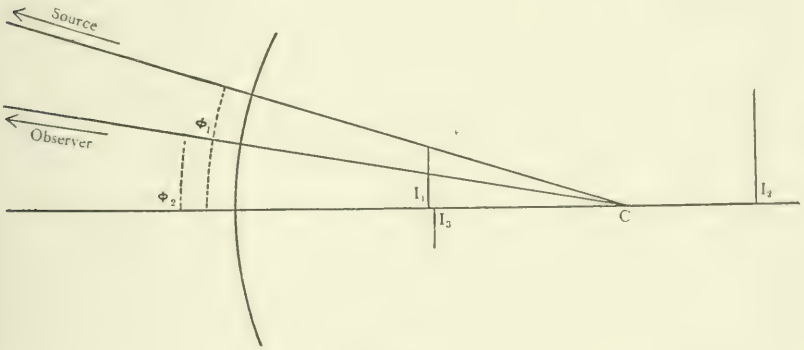


FIGURE 2.

The second condition was that the projections of the two points defined above fell together in both positions of the source and observer. These two conditions may be formally stated as follows:

Source on temporal side . . $\phi_1 = \psi - \delta\psi$, $\phi_2 = -\psi - \delta\psi$.
Source on nasal side . . . $\phi_1 = -\psi + \delta\psi$, $\phi_2 = \psi + \delta\psi$.

Substituting these values of ϕ_1 and ϕ_2 in the expression for the ordinates of the two projections, and setting them equal for each case, we have as the second condition:

Source on temporal side $0.0376\,\psi - 1.356\,\delta\psi = 0$.
Source on nasal side $-0.0376\,\psi + 1.356\,\delta\psi = 0$.

A zero value of $\delta\psi$ will not satisfy these equations, hence a perfectly symmetrical eye would have presented to Helmholtz just the phenomenon which he observed and any definite conclusion as to eccentricity is excluded.

The numerical coefficients of the above equations are, obviously, only correct for the schematic eye and are certainly different for all of the eyes observed. Moreover, the value of $\delta\psi$ would also depend upon any error of eccentricity or of collimation, if such errors were present. There is little to guide us in speculation as to this last point, since the value of ψ is not indicated; we only know that it was not a small angle, for then the brightness of I_1 would have rendered the other far fainter images invisible; on the other hand, ψ could not have been very large, or the iris would have hidden the fainter images. The average value of $\delta\psi$ in the above table is 1°, which would imply, in the schematic eye, a value of about 36° for ψ. This is so nearly what we should regard as a convenient value for such observations as described that the safest general conclusion would seem to be that these eyes were very free from the errors named.

THE DETERMINATION OF WATER IN FOODS AND PHYSIOLOGICAL PREPARATIONS.

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THE determination of water in materials of interest in physiological research may properly be divided into two operations. In the first place, in order to prepare the fresh material (which may frequently contain over 80 per cent of water) in a form to insure accurate sampling and correct weighing, it is necessary to expel the major portion of the water. Inasmuch as all desiccated material of this nature is prone to take moisture from the air, it is customary to stop the dehydration as soon as the material can be properly ground, and to rely on a new determination of water in the "partially dried" material to secure data regarding the absolute amount of water present. This paper has to do especially with the determination of water in the "partially dry" material; in general, with substances containing less than 20 per cent of water.

The demand of the technical analyst for a method that is rapid and approximately accurate has resulted in an almost universal adoption of the method of determining the water-content from the loss in weight of a given quantity of material when heated at about 100 C. Even the technical analyst has, however, frequently found it necessary to modify this general procedure; and when the method or any of its manifold modifications is applied to the analysis of the extremely complex substances necessarily employed in physiological chemical research, they are all found to possess numerous disadvantages that, though negligible in technical work, cannot be overlooked in the most accurate scientific research.

It is certainly erroneous to designate the loss in weight of a substance heated at 110 as water, when it can be distinctly shown that such heating causes a number of physical and chemical changes in the material, other than the simple loss of moisture, which also have an effect upon the weight. The indirect method of determining

water has, however, so very many advantages over the direct method that it is almost useless to consider any method involving the collection or absorption of water and the weighing of the quantity thus absorbed. Consequently, to render the indirect determination, with its numerous obvious advantages, more accurate, it is necessary to observe the loss in weight of the substance under conditions that insure, rather than assume, no variations in weight of the material due to causes other than loss of moisture.

Even at the temperature of boiling water many substances undergo profound changes under the influence of continued heat, and the continued action of heat and air is responsible for not inconsiderable alterations in weight. The early recognition of these secondary influences lead to numerous changes in the method for determining moisture-content.

By lowering the temperature and using a vacuum or some inert gas to avoid the effect of heated air, numerous investigators have determined conditions most suited for use with certain classes of materials. But it still remains a fact that no general method suitable for all materials is as yet in use, and consequently an accurate determination of water can only be secured by comparing the results of a number of methods; and even then it is by no means invariably obvious which method gives the true result.

The errors entering into the determination of water may be classed as follows.

1. Volatilization of material other than water.
2. Absorption of oxygen.
3. Abstraction of moisture from the air during the process of weighing.

Volatilization of material other than water. — The loss of material by volatilization has been studied in foods by Atwater,[1] Frear and Beistle,[2] and Cutter,[3] and in coal by Burk,[4] while numerous writers have observed the discoloration of concentrated sulphuric acid in which the direct absorption of water is frequently made.

The quantitative estimation of the loss of material by volatilization

[1] Atwater: Bulletin 21, United States Department of Agriculture, Office of Experiment Stations, 1895, p. 41.

[2] Frear and Beistle: Pennsylvania Experiment Station Report, 1902, pp. 165–168.

[3] Cutter: New York State Experiment Station, Bulletin 6, 1889, pp. 24 and 25.

[4] Burk: Journal of the American Chemical Society, 1898, xx, pp. 282–283.

has been carried out by three essentially different methods. In one the substance to be dried is heated in a closed vessel through which a current of dry carbon-dioxide-free air or gas is conducted. The issuing air containing the water-vapor and any volatilized material is conducted either over heated cupric oxide, and then through a solution of barium hydroxide, or through concentrated sulphuric acid or a solution of potassium permanganate. The degree of turbidity in the barium hydroxide solution, or discoloration of the sulphuric acid, or reduction of the permanganate solution, is taken as an indication of the amount of material volatilized.

The second method, assuming that all the volatilized material is fat or other material soluble in ether, consists in noting the loss in amount of ether-extract before and after prolonged heating of the material.

That either of these methods gives a reasonable approximation to the actual amount lost by volatilization is to be doubted; for in the first method the volatilized fats condense readily in the tubes conducting the gas and frequently do not come under the influence of either reagent; and in the second method, the quantity of ether-extract is very much diminished by heating in air, since the fats are rendered less soluble in ether by the absorption of oxygen. Furthermore, since the recent work of Lehmann,[1] it is obvious that the errors involved in the usual fat determination are too gross to permit the use of the variations in fat content as a check on the amount of material volatilized.

Both methods have been carefully studied in this laboratory, and we have lost confidence in their value save as indicating that there is a considerable loss of material by volatilization when the substances are heated at the temperature of boiling water.

The third method of studying the loss of material has to do with the loss of nitrogenous material only, and involves a determination of nitrogen in the substance before and after heating. That this loss may be large is readily seen from the data in the following table.

Two portions of fresh material were taken. One was dried in the ordinary way by heating at 100° in a water-jacket oven, and the other was dried in a vacuum-desiccator. The nitrogen was determined in the dried material.

[1] Lehmann: Archiv für die gesammte Physiologie, 1903, xcvii, p. 419.

TABLE I.

PER CENT NITROGEN IN ANIMAL FOOD AFTER DRYING IN HOT AIR AND IN DESICCATOR. (CALCULATED TO THE FRESH BASIS.)

Material.	Hot air.	Desiccator.
Beef *a*	4.86	5.13
Beef *b*	4.05	4.20
Chicken	4.19	4.52

These meats, therefore, after heating at 100° in a water oven lost from 4 to 7 per cent of the total nitrogen present.

Gibson, under Atwater's[1] direction, found similar losses in beef and fresh fish. In addition to determining the nitrogen in the fresh substance, and then in the material after drying at 100°, they dried the meat and fish in a current of dry hydrogen, conducted the gas into hot sulphuric acid, and determined subsequently the nitrogen in the acid. The quantity thus found agreed closely with the difference between the nitrogen of the fresh and that of the dried substance. They considered that the nitrogen thus lost was in the form of volatile amines.

It is thus evident that nitrogenous material in considerable amount may be lost during the process of drying, even if the temperature does not exceed that of boiling water.

In a special investigation on the method of the determination of solids in the analysis of vinegar, Frear and Beistle[2] found that, when vinegar was dried to constant weight in a water oven at 100°, 0.64 per cent more of the total weight of the vinegar was volatilized than when dried over sulphuric acid. "These results demonstrate beyond cavil the fact that the usual method of determining vinegar solids by evaporation at 100° C. is attended by the elimination, either by volatilization or decomposition, of constituents other than water, alcohol, and acetic acid, and that this loss of materials properly classed among solids is entirely too great to be overlooked in any investigation requiring an absolute determination of the quantity of such materials."

[1] ATWATER: *Loc. cit.*

[2] FREAR and BEISTLE: Pennsylvania State Experiment Station Report, 1902, pp. 165–168.

Absorption of oxygen. — That material is oxidized by heating in the air at 100° was early recognized, and numerous observers have had recourse to heating in an inert atmosphere of hydrogen, illuminating gas, or carbon dioxide. With many substances the oxidation is far too great to neglect even in technical analysis, and consequently for the analysis of many food materials for animals, especially those containing the drying oils and finely divided cellulose, specific directions are given to dry in hydrogen or illuminating gas.

A large number of experiments have been made in this laboratory in which various substances have been heated both in air and in the inert gases, especially hydrogen and carbon dioxide. The results of these experiments may be briefly stated as follows:

The use of an inert gas, as nitrogen, carbon dioxide, hydrogen, or illuminating gas, does materially prevent the absorption of oxygen and consequent gain in weight, but as the other great error affecting the water-determination, *i. e.*, volatilization of material, causes an error in the opposite direction, we believe that the use of an inert gas gives results too high, and consequently the heating at 100° in air probably gives on the average the closer approximation to the loss in weight that would correspond to the true per cent of water present. It is important, however, to recognize that the direct absorption of oxygen may materially affect the water-determination.

In a series of quantitative experiments, Schulz[1] found that heating lean fresh beef in an air bath at 110° caused a marked increase in the quantity of preformed sulphates in the meat, *i. e.*, from seven to ten times the quantity found in meat dried at 13° in a vacuum. While the absolute amount of sulphur thus oxidized is not great, the proof of oxidation is conclusive.

Errors in weighing. — When food products or physiological preparations are perfectly dried, and are transferred from a desiccator to the balance pan, a not inconsiderable increase in weight may take place during the process of weighing. The hygroscopic nature of desiccated vegetable or animal material cannot be lightly regarded in accurate work. The large surface usually presented by such material renders it especially prone to absorb moisture, and the use of a glass cover to the vessel containing the dried material has been advocated. This procedure minimizes the error, but we have found it advisable not to rely on the simple contact of a glass disk on the edge of a glass or metal dish, and in our work have used, instead

[1] Schulz: Archiv für die gesammte Physiologie, 1894, lvi, p. 203.

of this, aluminium dishes of special construction. It may be remarked that these dishes are used also in several other laboratories, and are giving excellent satisfaction.

The dish proper is of sheet aluminium, gauge 22, and is 55 mm. in diameter, with a depth of 15 mm. The internal diameter at the bottom is 52 mm., thus giving a slight flare to the sides. The cover fits into the taper of the dish like a plug, and is practically the counterpart of the dish itself, save that the sides are but 5 mm. high, and the external diameter at the bottom is slightly less than the internal diameter of the dish at the top. The short tapering side fits into the dish to about one-half the depth of the cover. A slight pressure serves to insert the cover, and if the latter is properly made[1] the cohesive nature of the aluminium insures its remaining in proper place. Such a stopper can be readily removed without any great effort, and with no danger of spilling the contents of the dish. The dishes, with the covers weigh about 11 gm. each, are easily kept clean, and are far more convenient than the glass dishes commonly used.

The error introduced in accurate work by not using covered dishes in weighing desiccated material is clearly seen when the gain in weight of such material is noted on the balance. An expert analyst, calculating as closely as possible the probable weight of the dish and substance, can rarely remove from the desiccator and weigh a sample accurately in less than one minute. During this time a sufficient quantity of moisture may be absorbed by the dried material to affect the water-determination by 0.05 to 0.1 per cent, for an increase in weight of 1 milligram causes an increase in the apparent per cent of water-free material of 0.05 per cent when, as is the case with all experiments reported in this paper, 2 gm. of material are used.

Furthermore the avidity for moisture of many desiccated materials, especially after desiccation in a high vacuum, is such that, when placed in an average desiccator, even over sulphuric acid, a not inconsiderable gain in weight will be observed on subsequent weighing, if the dishes have not been provided with covers.

Table II shows the increase in weight of four samples. In the first column of figures are given the actual weights of the aluminium dishes plus the weight of the dry matter in 2 gm. of the original

[1] A large number of these dishes have been made for us and for other laboratories by Messrs. Dinsmore and Singleton of Middletown, Connecticut.

sample. These dishes had all been subjected to a long desiccation in a high vacuum, and consequently the water-content was at a minimum. After removal from the high vacuum and weighing with covers on, they were placed in an ordinary desiccator over sulphuric acid, and allowed to stand with the covers off for five hours; they were then weighed with the covers on, and finally they were allowed to stand with the covers off for successive intervals of one minute on the balance pan, and the increase in weight noted. A determination of the moisture in the air of the balance-room at the time of this test showed that the air was about 60 per cent saturated with water-vapor. The temperature was 22° C.

TABLE II.

INCREASE IN WEIGHT OF DRY SAMPLES BY STANDING IN AIR.

Material.	Weight. Dry.	In ordinary desiccator. 5 hrs. without covers.	In air. Covers off.		
			1 minute.	2 minutes.	3 minutes.
	grams.	grams.	grams.	grams.	grams.
Beef	9.4480	9.4525	9.4537	9.4545	9.4557
Bread	9.3703	9.3748	9.3753	9.3761	9.3768
Fæces	13.1223	13.1277	13.1286	13.1295	13.1304
S. Wheat	13.1497	13.1535	13.1543	13.1549	13.1556

Much larger gains in weight than those noted in this table have frequently been observed, and our conclusion is that weights, to be trustworthy, must be in covered dishes, for experiment has shown that when contained in aluminium dishes with covers, such as we have described, desiccated vegetable or animal materials will not absorb more than 1 or 1.5 milligrams of moisture *per hour*, even if they are allowed to stand in the open air of the laboratory.

The relatively large area of material exposed in these dishes is, however, of considerable disadvantage in the most accurate work, and when the nature of the material to be weighed will permit, small glass-stoppered weighing bottles are to be preferred.

Effect of heat on vegetable and animal material. — Aside from its effect on the per cent of water, heat produces in many substances numerous alterations in composition that may not be sufficient to affect materially the weight, and yet are a decided disadvantage in studying the properties of vegetable or animal materials. The

charring of filter-paper and darkening of solutions containing sugar, as a result of heating at 100°, are well known. The partial dehydration of the levulose molecule[1] at 100° has necessitated modifications in the technical methods of analysis to avoid this difficulty.[2] That the proteid molecule may be considerably altered by heat is evidenced by the results of Volhard[3] on the solubility of the nitrogenous constituents of fodders in pepsin-hydrochloric acid, and by the cleavage of the casein molecule observed by Laqueur and Sackur.[4] Volhard compared the digestibility "in vitro" of material dried at 40°, 60° and 100°, and established that the digestibility of the nitrogenous constituents continually decreased, the higher the temperature at which the material is dried.

Laqueur and Sackur found that casein dried in a vacuum at 100° lost 0.8 per cent more weight than when dried in the open air, indicating, according to their opinion, a gain in weight by oxidation. They further found that casein dried at 100° either in vacuum or in air, was split by dilute alkalies into two products, the one soluble in dilute sodium hydroxide, and the other a jelly-like mass. This molecular change was, in their opinion, due to the effect of the heat used in drying the material.

These effects of heat may be, and generally are, produced in only a slight degree, though frequently they may interfere, perhaps in a hitherto unsuspected manner, with accurate observations of the properties of many substances.

Drying in vacuo vs. drying by heat. — Because of the deleterious effect of heat on many compounds, a partial vacuum has frequently been used in the effort to minimize this effect. However, the demands of technical analysis have, as a rule, interdicted the use of the vacuum, owing to the increased time required for desiccation, though in certain special methods of analysis the use of a vacuum has been successfully employed.

The vacuum method has been studied with cattle foods by Knorr;[5]

[1] Wiley: Principles and Practise of Agricultural Analysis, iii, p. 19.

[2] Thorne and Jeffers: Journal of the Society of Chemical Industry, 1898, xvii, pp. 114–116; Shutt and Charbon: Transactions of the Royal Society, Canada, 1902–3, second series, 8, iii, pp. 35–46.

[3] Volhard: Die landwirthschaftliche Versuchsstation, 1903, lv, Parts V and VI.

[4] Laqueur and Sackur: Beiträge zur chemischen Physiologie und Pathologie, 1903, iii, p. 193.

[5] Knorr: United States Department of Agriculture, Division of Chemistry, 1890, Bulletin 28, pp. 82–85.

with sugar solutions, honey, etc., by Carr and Sanborn,[1] Thorne and Jeffers,[2] and Shutt and Charbon;[3] with meat and fish by Atwater and Gibson;[4] with starch by Dafort;[5] and with coals by Langbein[6] and Hillebrand.[7]

It is probably true that drying "in vacuo," as usually described, should read "in partial vacuo." As will be shortly pointed out, enormous differences in rapidity and completeness of desiccation depend solely on the variations in pressure inside the desiccator.

In some instances the partial vacuum employed was accompanied by heat either at 100° C., or generally with a somewhat lower temperature. Special forms of apparatus like those of Carr[8] or Langbein[9] permit of wide variations in the temperature and pressure, but do not admit of very complete exhaustion. In Carr's apparatus the tension of the residual gas is seldom less than five inches of mercury, and with a temperature of 70° in the water jacket it is calculated that the substance is being heated 13° above the boiling point of water, since the tension of aqueous vapor at 56.3° is equal to 125.07 mm. (five inches) of mercury.

It is obvious that, the higher the vacuum, the lower the boiling point of water, and consequently the more rapid the desiccation. With the apparatus ordinarily at hand, *i. e.*, a vacuum-desiccator and a water suction-pump, it is seldom possible to obtain a vacuum of more than 20–30 mm. of mercury; and, as ordinarily manipulated, it is doubtful if a desiccator with a vacuum of 60 mm. will maintain this degree of rarefaction for any length of time. We wish to emphasize the importance of using a manometer *inside the desiccator* in all work in which a vacuum is employed. We believe that in no other way can one be sure of the pressure conditions inside the desiccator from hour to hour. A 150 mm. length of small glass tube sealed at one end, bent in a U shape, and the closed arm completely

[1] Carr and Sanborn: United States Department of Agriculture, Division of Chemistry, 1896, Bulletin 47, pp. 134–152.

[2] Thorne and Jeffers: *Loc. cit.*

[3] Shutt and Charbon: *Loc. cit.*

[4] Atwater and Gibson: *Loc. cit.*

[5] Dafort: Zeitschrift für analytische Chemie, 1896, xxxv, p. 622.

[6] Langbein: Zeitschrift für angewandte Chemie, 1900, p. 1232.

[7] Hillebrand: Journal of the American Chemical Society, 1898, xx, pp. 282–283. See also Journal of the American Chemical Society, 1899, xxi, pp. 1116–1134.

[8] Carr: *Loc. cit.*

[9] Langbein: *Loc. cit.*

filled with clean mercury will answer every purpose. Equipped with this simple manometer, it will be found that few water suction-pumps will give a vacuum of much less than 20 mm. The degree of exhaustion is dependent on the water-pressure, temperature of the water, efficiency of the water-pump, and the construction (especially the ground glass joints) of the desiccator. With otherwise ideal conditions, the rarefaction can never be less than the tension of aqueous vapor at the temperature of the water used in the suction-pump. In order to be most efficient for desiccation, however, it is essential that the tension of the rarefied gas in the desiccator be somewhat less than the tension of aqueous vapor at the room-temperature. This degree of rarefaction has hitherto only been obtained by the use of the complicated, costly, and fragile mercury-pump. Owing to the length of time required to exhaust a vessel of the size of an ordinary desiccator by means of the mercury-pump, no use of high vacua has been made in drying operations, save in a few isolated cases.

The chemical method described by us in a previous paper[1] has, however, furnished the means for the production of high vacua rapidly, economically, and with the use of no special apparatus, and we believe that drying in high vacua *without the aid of heat* has now a new claim to consideration.

With the absence of heat there is no loss by volatilization of material other than water, and the intermolecular changes frequently induced by heat are avoided, while the absence of air precludes any oxidation. The one objection is that the time required to secure complete desiccation is considerably longer than that required when the material to be dried is heated in the usual manner. Believing that, however much the technical analyst must sacrifice absolute accuracy to rapidity of determination, analyses made in connection with scientific research should be as accurate as possible, we have studied the desiccation in high vacua with the idea of suggesting its use in most accurate work.

Manipulation of vacuum-desiccator. — The method of producing a high vacuum in a Hempel desiccator depends on the fact that the air in the desiccator is first expelled by ether vapor, and the residual ether vapor is then absorbed by concentrated sulphuric acid. A vacuum of less than 1 mm. of mercury can thus be obtained in a Hempel desiccator containing 2.5 litres of air in ten minutes.

[1] American Chemical Journal, 1902, xxvii, p. 340.

The details of manipulation in connection with the determination of water in samples are as follows:

The samples (generally 2 gm.) are weighed in aluminium dishes with covers, usually in duplicate. The desiccators commonly used are of the Hempel form, although the Shiebler desiccator, with the removable acid dish permitting the use of the acid in the upper portion of the desiccator, has been used with equal success.

To economize space and allow the maximum number of dishes in each desiccator, it is advisable to have a rack made with shelves of perforated metal, the shelves to be just far enough apart to permit convenient removal of the aluminium dishes. A brass tube, 3 mm. internal diameter, open at both ends, is soldered in the centre of the rack extending through the top shelf to just flush with the bottom of the lowest shelf. Such a rack will hold from sixteen to thirty aluminium dishes. A ring soldered at a convenient part of the top shelf aids in removing the rack from the desiccator. At one side of the rack it is convenient to fasten the simple manometer described on page 317, which is absolutely essential to the success of the method. As the dishes are placed in the rack, the covers are removed, and for convenience each dish is loosely set in the taper of its cover. The rack is then placed in the perfectly clean, dry desiccator, fresh acid (100–200 cc. of concentrated, commercial H_2SO_4, 1.84 specific gravity) placed in the acid compartment, and the whole apparatus carefully connected with a water suction-pump. To prevent "back suction," we invariably place an empty Drechsel gas washing-bottle between the desiccator and the pump, connecting the pump with the tube extending to the bottom of the bottle by means of thick-walled rubber tubing that will not readily collapse. It is well to coat the stopper of the gas washing-bottle with a layer of vaseline, or better a mixture of beeswax, Venice turpentine, and vaseline. The thicker waxes do not draw into the bottle under greatly diminished pressure as does vaseline. The stopcock on the desiccator, as well as the edges of the cover, should be well covered with a thin layer of vaseline, or the mixture suggested. The cover should be removed, or slid to one side for a moment, and 10 cc. of ether delivered from a dry pipette into the upper end of the brass tube extending through the rack. The cover is immediately replaced and the water-suction started. After the exhaustion has commenced the beeswax-vaseline mixture is carefully applied around the edge of the cover. The use of this wax has proven unusually

successful in securing a tight closure around the edges of the desiccator and stopcock. It is easily prepared by melting together 60–75 gm. of beeswax, 15 gm. Venice turpentine, and 15 gm. of vaseline. The proportion of beeswax is varied with the temperature of the laboratory. In winter, less beeswax will suffice than in summer.

If the delivery tube of the suction-pump dips under water, with an average pump air-bubbles will cease to rise in appreciable quantities in about four to five minutes (with a desiccator containing 2.6 litres). The ether, which can be seen on the bottom of the desiccator, soon begins to boil, and in a few minutes the mercury levels in the manometer begin to approach each other. When the difference in level is about 35 mm. the stopcock is closed, the rubber tubing disconnected, and then the suction-pump is stopped. The heavy ether vapor expels all air, and the vapor left in the desiccator when the stopcock is closed is absorbed by the concentrated sulphuric acid, producing a vacuum of less than 1 mm. of mercury in about eight to ten minutes.

If the stopcock and cover of the desiccator have been well fitted and well lubricated with wax, the vacuum will hold indefinitely. The desiccator is now placed where it will not be disturbed, preferably in a temperature not above 20° C., and left until the materials are dry. The length of time necessary for this operation is in general about two weeks.[1] The manometer should be observed occasionally, and if a leak occurs the desiccator should be exhausted again.

In order to minimize the absorption of moisture from the atmosphere by the dried material on opening the desiccator, we connect the stopcock of the desiccator, by means of a rubber tube, with the exit tube of a Drechsel gas washing-bottle containing concentrated sulphuric acid, and allow the dry air to enter the desiccator through the acid till the pressure becomes atmospheric. A sufficient number of small ordinary desiccators to hold all the aluminium dishes being previously arranged in a convenient place, the cover of the vacuum-desiccator is removed, the rack and dishes withdrawn, the cover of each dish firmly and rapidly pressed into place and the dish placed in one of the smaller desiccators. With a little skill, one can transfer ten to twenty dishes in this way in a few moments, and no appreciable amount of moisture will be absorbed. The dishes are then imme-

[1] For detailed data on this point, see page 322.

TABLE III.

COMPARATIVE DESICCATION IN AIR, HYDROGEN, AND A VACUUM (PER CENT OF WATER).

Material.	Heat. 100° C. — 5 hours.		In vacuum at room-temperature (0 — 5 mm. pressure).										
	Air.	Hydrogen.	23 hours.	50 hours.	70 hours.	1 week, 2 days.	2 weeks.	2 weeks, 3 days.	3 weeks.	4 weeks.	6 weeks.	7 weeks.	11 weeks.
Composite . . .	3.73	4.08	1.37	..	2.02	..	3.11						
Gingersnaps *a* . .	5.24	5.71	2.96	..	3.77	..	4.93						
Shredded wheat *a*	7.98	8.76	6.66	..	7.57	..	8.48						
Beef	3.27	3.47	..	3.17	..	3.37	3.44	3.42	3.38	3.41	3.37	3.34	3.30
Bread	6.07	6.54	..	4.86	..	5.78	6.00	6.07	6.15	6.29	6.40	6.43	6.60
Fæces	6.32	6.68	..	4.66	..	5.26	5.39	5.40	5.42	5.52	5.53	5.55	5.62
Gingersnaps *b* . .	5.63	5.89	..	4.24	..	5.02	5.20	5.21	5.30	5.39	5.38	5.28	5.36
Shredded wheat *b*	8.61	8.98	..	6.91	..	7.96	8.23	8.26	8.41	8.59	8.77	8.82	8.99

diately weighed. The details of manipulation may seem trifling, but experience has shown that this routine is decidedly advantageous.

Drying experiments with a vacuum-desiccator. — A number of materials of widely varying nature have been subjected to the drying operation at 100° in air, at 100° in hydrogen, and in high vacua (from 0 to 5 mm. mercury) over sulphuric acid, and the comparative results are given in Table III. The drying in air and hydrogen at 100° was continued for five hours. The desiccation in vacuo continued for varying length of time, as indicated by the columns in the table. As stated before, 2 gm. of material were used in each case, and consequently a variation of 0.1 per cent in the apparent percentage of water present means a change in weight of but 2 milligrams. The results are invariably from duplicate determinations. The agreement of duplicates was striking in all cases.

It is seen that there are marked discrepancies between the percentage of water as determined by heating and by desiccation, and furthermore the percentage as determined by drying in hydrogen is invariably greater than that by drying in air. It is reasonable to suppose from these data that the loss in weight by heat is a resultant of three factors: first, loss in weight of water; second, volatilization of material other than water; third, oxidation. To these three factors may be added the possibility of the actual splitting off of water from the proteid or carbohydrate molecule, such as, for example, the formation of an anhydride.

A number of experiments were made on materials of vegetable and animal nature to observe the effect of prolonged drying in a high vacuum over sulphuric acid. Comparative tests with heat were not made. The results of the animal materials are given in Table IV, and those for the vegetable materials in Table V.

The materials of animal origin include a number of albuminoids[1] which were under investigation at the same time in this laboratory.

With these animal materials it is seen that, in general, the drying operation is complete at the end of two weeks, and subsequent desiccation is without material effect on the absolute moisture-content. This is nowhere more markedly shown than in the case of the albuminoids that were kept in a high vacuum for six months. The subsequent loss in weight after the first two weeks was inconsiderable in all cases save one. It is to be regretted that weighings were not

[1] For these preparations we are indebted to Professor WILLIAM J. GIES of Columbia University.

TABLE IV.

DESICCATION OF ANIMAL MATERIALS IN A HIGH VACUUM (0 — 5 MM. MERCURY) (PER CENT OF WATER).

Material.	23 hrs.	50 hrs.	70 hrs.	1 wk.	1 wk. 2 da.	2 wk.	2 wk. 3 da.	3 wk.	4 wk.	6 wk.	7 wk.	11 wk.	6 mo.
Beef *a*	4.61	..	4.89	..	..	5.11							
Chicken	..	..	..	..	2.88	2.91	..	2.94					
Codfish	..	..	..	..	2.70	2.79	..	2.79					
Duck	..	..	..	..	0.53	0.57	..	0.71	0.71				
Salmon	..	..	..	..	0.97	1.10	..	1.27	1.30				
Beef *b*	..	3.17	..	..	3.37	3.44	3.42	3.38	3.41	3.37	3.34	3.30	
Butter	..	..	12.39	..	12.43								
Fæces *a*	2.04	..	2.47	..	..	3.14							
Fæces *b*	..	4.66	..	..	5.26	5.39	5.40	5.42	5.52	5.53	5.55	5.62	
Collagen and elastin Femur II	..	..	..	10.61	..	10.64	..	..	10.60				
Collagen and elastin Rib IV	..	..	..	11.42	..	11.50	..	..	11.52				
Ossein Femur I	..	..	..	11.66	..	11.74	..	..	11.77				
Gelatin Femur B	..	..	..	11.85	..	11.90	..	..	11.95				
Collagen and elastin Femur III	..	..	..	12.29	..	12.43	..	..	12.47				
Collagen Tendon C	..	..	..	12.98	..	13.09	..	..	13.10				
Elastin Ligament D[m]	..	..	..	7.13	..	7.24	..	..	7.23				
Ossein Femur V	..	..	..	9.95	..	10.16	..	..	10.25				
Collagen and elastin Femur	..	..	..	..	..	13.49	..	..	..	..	..	..	13.59
Collagen Tendon	..	..	..	..	..	14.62	..	..	..	..	..	..	14.86
Collagen Tendon	..	..	..	..	..	15.75	..	..	..	..	..	..	16.15
Collagen Tendon	..	..	..	..	..	15.04	..	..	..	..	..	..	15.27
Collagen Tendon	..	..	..	..	..	15.43	..	..	..	..	..	..	15.57
Collagen Femur	..	..	..	..	..	14.27	..	..	..	..	..	..	14.35
Gelatin Tendon	..	..	..	..	..	12.92	..	..	..	..	..	..	13.06
Ossein Femur	..	..	..	..	..	13.61	..	..	..	..	..	..	13.46
Ossein Femur	..	..	..	..	..	13.59	..	..	..	..	..	..	13.71
Ossein Femur	..	..	..	..	..	17.93	..	..	..	..	..	..	18.08
Collagen Femur	..	..	..	..	..	16.73	..	..	..	..	..	..	16.76

TABLE V.

DESICCATION OF VEGETABLE MATERIALS IN A HIGH VACUUM (0 — 5 MM. MERCURY)
(PER CENT OF WATER).

Material.	23 hours.	50 hours.	70 hours.	1 week, 2 days.	2 weeks.	2 weeks, 3 days.	3 weeks.	3 weeks, 3 days.	4 weeks.	6 weeks.	6 weeks, 4 days.	7 weeks.	11 weeks.
Composite . . .	1.37	..	2.02	..	3.11								
Cotton-seed meal	7.64	..	8.27	..	8.79								
Gingersnaps *a* . .	2.96	..	3.77	..	4.93								
Gluten crackers .	7.54	..	8.31	..	8.96								
Hay	7.09	..	7.30	..	7.48								
Shredded wheat *a*	6.66	..	7.57	..	8.48								
Bread	..	4.86	..	5.78	6.00	6.07	6.15	..	6.29	6.40	..	6.43	6.60
Gingersnaps *b* . .	..	4.24	..	5.02	5.20	5.21	5.30	..	5.39	5.38	..	5.28	5.36
Shredded wheat *b*	..	6.91	..	7.96	8.23	8.26	8.41	..	8.59	8.77	..	8.82	8.99
Edestin	..	..	..	5.64	..	5.76	..	5.95	..	..	5.99	..	

made at the end of three weeks with this set of samples, for probably the major portion of the slight loss took place during the third week.

The complete dehydration of butter in a high vacuum is of especial interest, when we consider the difficulties usually experienced in securing an equal distribution of the material in the sample dish and the passage of the water through the supernatant layer of melted fat. It is worthy of note that in several tests made with the vacuum method no especial degree of importance could be ascribed to an even distribution of the sample. Desiccation was equally complete whether the sample was first melted in a thin layer on the bottom of the dish, or large irregular lumps of butter were used.

As seen in Table V, with vegetable materials we find a continued slight loss for several weeks, and while the loss in weight during the first two weeks is probably as close a measure of the absolute amount of water present as is the loss in weight after heating at 100° for five hours, nevertheless, the continued slight loss in weight in a vacuum signifies that moisture is still present.

The cereal products, bread and shredded wheat, show this continued loss most markedly, while singularly enough the sample of gingersnaps does not exhibit the water-retaining property of the other two.

Fæces were included under the head of animal materials, since, in all probability, according to Prausnitz, the fæces consist more of metabolic products, *i. e.*, biliary residues, intestinal débris, etc., than of undigested vegetable material. As a matter of fact, the sample of fæces here used was that from a man living essentially on a vegetable diet. It is obvious that the large differences between the per cent of water as found by the two methods with heat and the third method by vacuum (Table III) is attributable to the volatilization of large amounts of material other than water.

The length of time required to secure complete desiccation in a vacuum is perhaps the most interesting feature of these results. Obviously, a marked difference exists between materials of a vegetable and those of an animal nature, the former retaining their moisture persistently while the latter have, in general, lost all but mere traces of their moisture after two weeks in a high vacuum. For animal materials, therefore, we may say that two weeks in a high vacuum suffices to remove practically all moisture. For the most accurate work a longer time is to be recommended, though

the slight loss in weight subsequent to two weeks' desiccation can hardly affect ordinary physiological or chemical research.

With vegetable materials, desiccation should proceed for a period longer than two weeks. As a matter of fact, however, it can readily be seen that the sample, as soon as prepared for analysis, can be placed in the vacuum and weighed as soon after two weeks as the results are needed. In general, the time between the preparation of a sample for analysis, and the time when the results are imperatively needed, is considerably over two weeks. For preliminary determinations in metabolism experiments, where an approximate knowledge of the water-content of foods is necessary, the usual five-hour heating will suffice, and the final calculations may be based on the determination by the vacuum method. For technical work, obviously the method is, as already stated, too time-consuming, though the advantages may appeal to the technical analyst and the method may possibly find some use in special cases.

Experiments on the absorption of water by desiccated material. — The marked difference in time required to remove moisture completely from animal and vegetable material suggested the idea that in materials of a vegetable origin the structure of the cell-walls might be such as to retard considerably the evaporation of water. Were this the case, we should expect that after the samples had once become thoroughly dry, moisture subsequently absorbed would be retained in a different manner, and in all probability more easily given up in a high vacuum. Samples of beef, bread, fæces, gingersnaps, and shredded wheat were removed from the high vacuum in which they had been placed several weeks before. After weighing, they were exposed with the covers off to air saturated with water vapor at 30° for two and one-half hours. The gain in weight was marked in all cases. The samples were then placed immediately in a high vacuum, and weighed at the end of eighteen, forty, and one hundred and twelve hours, respectively. The results expressed in percentages of moisture found are given in Table VI.

The first column of figures gives the per cent of water actually found as a result of desiccation in a high vacuum for several weeks. The second column indicates the per cent of water in the materials after being exposed for two and one-half hours to an atmosphere saturated with water-vapor at 30° C. These figures are obtained from the weight of the dried material and the gain in weight, *i. e.*, water absorbed. It is thus seen that much more water was absorbed

than was originally in the sample. The remaining three columns show the per cent of moisture found on weighing the samples at the stated intervals. Obviously the figures in these columns should tend to approach those in the first column. That they actually do is seen from an inspection of the table. The remarkable fact about the experiment is that the water thus absorbed appears to be as persistently retained as that originally present in the sample, thus indicating an unusually powerful hygroscopic nature as a property of these products.

TABLE VI.

DESICCATION OF MATERIALS AFTER EXPOSURE TO MOIST AIR.

Material.	Per cent water.				
	Original.	2½ hours in oven.	18 hours in desiccator.	40 hours in desiccator.	112 hours in desiccator.
Beef	3.31	10.44	2.98	3.23	3.28
Bread	6.28	11.43	3.52	4.28	5.04
Fæces	5.24	9.37	4.33	4.76	5.06
Gingersnaps . . .	5.34	9.91	3.00	3.78	4.54
Shredded wheat .	8.77	9.63	6.32	7.05	7.76

Comparative results in desiccators with varying degrees of rarefaction. —A series of experiments was made in which duplicate sets of samples of similar nature were dried in desiccators over sulphuric acid with degrees of rarefaction ranging from atmospheric pressure to a vacuum of less than 1 mm. of mercury. Four Scheibler desiccators with the acid dish at the top were used in this test. Each was provided with a rack on which were placed ten aluminium dishes containing the five pairs of samples. The pressures were atmospheric, half an atmosphere, 86 mm. of mercury, and the high vacuum. The desiccators were opened regularly with all due precautions, and the dishes containing the samples weighed. The results are shown in Table VII.

The table shows clearly that the efficiency of dehydration in a desiccator at atmospheric pressure is considerably increased when the pressure has fallen to 86 mm. On increasing the degree of rarefaction to less than 1 mm. of mercury, however, the increased efficiency in desiccation is very marked.

This is easily explained by the fact that the tension of aqueous vapor at the laboratory temperature (20° C.) is about 17 mm. of mer-

TABLE VII.

DESICCATION WITH VARYING DEGREES OF RAREFACTION (PER CENT OF WATER).

Material.	In desiccator.										
	Pressure.	2 da.	1 wk. 2 da.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	11 wk.
Beef	Atmospheric	1.24	2.15	2.47	2.62	2.66	2.69	..	..	2.83	
	½ atmosphere	1.76	2.47	2.72	2.80	2.81	2.92	..	..	3.03	
	86 mm.	2.05	2.73	2.92	3.01	3.06	3.09	..	..	3.21	
	0–5 mm.	3.17	3.37	3.44	3.38	3.41	..	3.37	3.34	..	3.30
Bread	Atmospheric	3.02	4.67	5.16	5.42	5.50	5.56	..	..	5.80	
	½ atmosphere	3.90	5.05	5.48	5.62	5.73	5.86	..	..	6.06	
	86 mm.	4.06	5.32	5.68	5.81	5.96	6.05	..	..	6.26	
	0–5 mm.	4.86	5.78	6.00	6.15	6.29	..	6.40	6.43	..	6.60
Fæces	Atmospheric	2.31	3.49	3.95	4.15	4.25	4.30	..	..	4.58	
	½ atmosphere	2.95	4.00	4.29	4.37	4.51	4.63	..	..	4.74	
	86 mm.	3.26	4.28	4.60	4.72	4.81	4.93	..	..	5.14	
	0–5 mm.	4.66	5.26	5.39	5.42	5.52	..	5.53	5.55	..	5.62
Gingersnaps . .	Atmospheric	3.02	4.12	4.48	4.68	4.73	4.80	..	..	5.00	
	½ atmosphere	3.56	4.49	4.75	4.85	4.94	5.03	..	..	5.19	
	86 mm.	3.68	4.67	4.95	5.02	5.14	5.21	..	..	5.38	
	0–5 mm.	4.24	5.62	5.20	5.30	5.39	..	5.38	5.28	..	5.36
Shredded wheat	Atmospheric	5.22	6.95	7.54	7.82	7.87	8.00	..	..	8.28	
	½ atmosphere	6.11	7.51	7.84	8.02	8.14	8.30	..	..	8.50	
	86 mm.	6.32	7.65	8.06	8.24	8.34	8.50	..	..	8.70	
	0–5 mm.	6.91	7.96	8.23	8.41	8.59	..	8.77	8.82	..	8.99

cury, and consequently a pressure of 86 mm. requires a considerably higher temperature to cause the tension of aqueous vapor to equal that of the gaseous medium. On the other hand, with the high vacuum, the temperature of the room is perhaps 20° above the boiling point of water at this degree of rarefaction, and consequently

evaporation of water proceeds with great rapidity. A consideration of these facts explains the slow action of vacuum-drying as usually carried out, and also accentuates the importance of using a manometer inside the desiccator to indicate the pressure conditions after exhaustion.

The desirability of using a partial vacuum with heat somewhat below 100° is in the light of the above consideration as to temperature and vapor-pressure called very much into question. The method for obtaining high vacua, as above outlined, requires no complicated or costly apparatus, and after exhaustion the desiccator may be placed on a shelf and left untouched, save for an occasional inspection of the manometer, until the determination is needed.

All vacuum apparatus for temperatures above that of the room, with which we are familiar, are complicated, readily get out of order, and require considerable attention. We have made a number of experiments with desiccators under high vacuum by placing them in a water bath the temperature of which could be controlled, but the lubricant melts, is easily drawn into the desiccator by reason of the vacuum, and soon the manometer indicates a leak. The use of higher melting lubricants is hardly to be recommended, for the covers are apt to stick, and with the acid chamber at the top, there is danger of spilling acid on attempting to open the desiccators. That a combination of the high vacuum and a high temperature (50°–60°) can be accomplished we do not deny, but the desirability of complicating a process so simple and successful seems to us, at the moment of writing, of questionable advantage.

PHARMACOLOGY OF ETHYL SALICYLATE.

By E. M. HOUGHTON.

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SINCE it has been so thoroughly established that methyl alcohol is much more toxic than ethyl alcohol, when administered for some time (Hunt), it seemed desirable to make a pharmacological study of the corresponding salicylates, particularly the ethyl compound. On careful search of the literature on the subject I was unable to find that any one had studied the pharmacological properties of ethyl salicylate (salicylic ethyl ester). Likewise, there were very few chemical data available, only here and there a statement that it had been prepared or could be prepared in a similar way as the methyl ester.

In view of these facts it seemed desirable to make a systematic study of the pharmacology of the drug, comparing the results with those obtained by the use of the well-known methyl salicylate, oil of wintergreen, and oil of birch. It should be stated that the experiments mentioned in this report were made with a specially prepared, very pure sample of ethyl salicylate[1] and natural methyl salicylate (oil of birch).

Ethyl salicylate is a transparent, colorless, volatile, slightly soluble, oily fluid.

Antiseptic action. — To a number of tubes of beef bouillon a large excess of ethyl salicylate was added, and the tubes thoroughly shaken in order to completely saturate the fluid. The tubes were then inoculated with cultures of bacillus pyocyaneus, and again shaken thoroughly, a few drops of the oil remaining in the bottom of the tube, then placed in an incubator for twenty-four hours, when they were taken out and examined. Growth was found in all of the tubes, but it was less than in the control tubes. Subsequent retests showed conclusively that the fluid is not an antiseptic, but that it slightly retards growth.

[1] It is with pleasure that I acknowledge my indebtedness to my colleague, Mr. C. P. Beckwith, for calling my attention to ethyl salicylate and for making a suitable product for experimental purposes. — E. M. H.

Odor and taste. — Ethyl salicylate possesses a pleasant characteristic odor and taste, somewhat similar to, but less pronounced than, the methyl compound.

Local action. — Ethyl salicylate applied to the tongue or fauces produces a slight burning sensation, but on the mucous membrane on the inside of the lips no irritation was noticed; while in both cases irritation followed the application of the methyl salicylate. When applied to the skin, and covered with a dressing to prevent evaporation, no redness or other evidence of irritation was manifested. A number of guinea-pigs were injected subcutaneously with large quantities of the drug. From four to twenty hours later most of the animals were killed, and post-mortem examination showed little evidence of irritation, but the tissues for some distance around the point of injection were bloodless and filled with droplets of unabsorbed oil. The appearance somewhat resembled that found after an application of carbolic acid, but complete absorption finally occurred in several animals kept alive without permanent damage. Enough of the oil was absorbed to show in a couple of hours the general toxic effect of the drug.

Toxicity. — A number of experiments were made to determine the toxicity and irritability of ethyl salicylate:

a. Minimum fatal dose per gram body-weight was determined for guinea-pigs, as frogs were found unsatisfactory, on account of the insolubility of the drug. The following table shows the toxicity per os to be 0.0014 gm. per gram body-weight:

TABLE I.

Guinea-pig.	Weight of pig.	Dose per gram body-weight.	Total dose.
		gram	
1	440	0.002	0.88 dead.
2	472	0.003	1.42 "
3	460	0.004	1.84 "
1	430	0.001	0.43 alive.
2	447	0.0015	0.67 dead.
3	442	0.002	0.89 "
1	395	0.0012	0.47 alive.
2	420	0.0013	0.55 "
3	380	0.0014	0.53 dead.
4	360	0.0014	0.50 "

The minimum fatal dose of the methyl compound similarly determined was 0.0007 per gm. body-weight. A second series of pigs showed substantially the same results.

b. The minimum fatal dose of ethyl salicylate when injected subcutaneously was 0.0015 per gram body-weight, or about the same as when given per stomach.

c. Several dogs were given large doses of ethyl salicylate on successive days to determine the effect of continuous dosage. More or less gastric irritation, as evidenced by nausea and vomiting, was produced. The act of emesis seemed to be a sort of regurgitation, as very little nausea and retching was observed.

The following is a typical experiment. The ethyl salicylate was given in soft gelatin capsules (globoids) undiluted.

Dog No. 4. — Weight 9.5 kg.

1st day. 5 c.c. Emesis one hour later, otherwise not much affected.

2d day. 5 c.c. Seems to be in normal condition. Emesis two hours later, otherwise but slightly affected.

3d day. 10 c.c. Seemed to be in normal condition. Emesis about two hours later. Sleepy.

4th day. 10 c.c. Seemed in normal condition. Did not vomit, but became very sleepy. Does not eat well.

5th day. 10 c.c. Seemed in normal condition. Did not vomit, and is becoming emaciated.

6th day. 10 c.c. Seemed in normal condition. Did not vomit. Became sleepy. Diarrhœa.

7th day. 10 c.c. Dog does not eat much, and is becoming much emaciated. Diarrhœa pronounced.

8th day. 20 c.c. Dog not eating well, and is becoming quite feeble. Vomited about half an hour later. Less diarrhœa.

9th day. 10 c.c. Dog has become quite weak. Emesis two hours later. Diarrhœa again becomes marked.

10th day. Experiment discontinued, and the animal regains his normal condition a few days later. During the nine days while under treatment the animal's weight decreased to 7 kg., — a loss of 2.5 kg.

Experiments were undertaken to determine the rate of absorption and excretion of ethyl salicylate.

a. Intravenous injection of small amounts of a mixture of 10 per cent ethyl salicylate and 50 per cent alcohol into dogs. Tube tied in ureter and the urine tested drop by drop with ferric chloride as it flowed from the cannula. It was found that usually in about ten minutes the urine became darkened, and gave the characteristic phenol reaction. Likewise, the urine, where large quantities of either the ethyl or methyl salicylate was injected, quickly became

bloody, showing quite conclusively that these compounds in their concentrated form were liable to produce irritation of the kidney.

b. A chloretonized dog, supplied with artificial respiration, received in the air-current strong vapors of ethyl salicylate continuously for thirty minutes. Tests of the urine were made from time to time for two hours, but with negative results.

c. In all cases the flow of urine was retarded, the number of drops from minute to minute being recorded on a kymograph by an electric current which was opened and closed by means of a delicate, inclined lever, which, when depressed by the weight of a drop of urine, closed the circuit, the lever being drawn up by a spring, and the current broken as the drops flowed off.

d. Seven men offered themselves as subjects for experiment. Each took at 9 A. M., about two hours after breakfast, 1 c.c. of ethyl salicylate dropped on a cube of sugar, and a half litre of water was drunk immediately afterwards. In each instance the bladder was emptied just before the experiment was commenced, and urine was voided every fifteen minutes afterwards, until a distinct reaction to ferric chloride was obtained, indicating the presence of salicylic compounds in the secretion. In four instances the reaction was obtained in thirty minutes, and in the other three, forty-five minutes. Repeated tests were made for the rest of the day up to 6 P. M., a salicylic reaction being always obtained, but by the following morning the urine had become normal. The experiments were repeated on the following day, 1.5 c.c. of the ethyl salicylate being given. These results were substantially the same, except in one instance, when a positive reaction was obtained fifteen minutes after the drug was taken.

Three men two days later took 1 c.c. each of the drug at 4 P. M. A reaction was promptly obtained. The following morning reactions were obtained until about 10 A. M., when the reaction was so faint as to be questionable.

None of the men experienced irritation of the stomach except a feeling of warmth or other untoward symptoms from the drug, and there was no apparent change in the urinary secretion except the characteristic darkening that occurs when any salicylate is given.

e. Small spots on the backs of two dogs were shaved and ethyl salicylate applied and thoroughly rubbed into the skin. No evidence of excretion in urine was found. Likewise, negative results were obtained when it was applied to the human integument for several hours in succession and covered to prevent evaporation.

Ethyl salicylate then, as well as the methyl compound, produces some irritation of the kidney when given intravenously in the form of an emulsion with alcohol. It is not absorbed from the respiratory tract or skin or, at least, it is absorbed very slowly, but it is rapidly absorbed from the stomach and is excreted through the kidney, presumably in the same way as the other salicylates. Excretion begins within half an hour after the drug is taken, and is completed in about eighteen hours.

TABLE II.

Time.	Temperature. Degrees F.				Pulse-rate.				Respiration-rate.			
	1.	2.	3.	4.	1.	2.	3.	4.	1.	2.	3.	4.
P. M.												
1.20	101.2	102.8	101.9	101.0	96	102	92	100	22	20	22	23 N
1.30	101.6	102.8	101.7	101.0	94	100	94	84	20	26	20	20 N
2.00		Dogs Nos. 1 and 3 each received 10 c.c. ethyl salicylate. Dogs Nos. 2 and 4 each received 15 c.c. ethyl salicylate.										
2.45	101.5	102.3	101.0	100.7	84	102	96	84	18 D	16	16	14
3.15	101.4	103.0	101.0	101.3	92	110	90	94	28	72 VD	22 S	18 VD
3.50	101.5	103.4	101.3	102.2	92	120	92	98	26 V	70 D	152 VP	34
4.15	101.8	103.2	102.2	102.5	80	136	100	102	24	62 V	145 P	36 V
4.45	101.6	102.0	102.0	102.0	100	124	108	110	38 P	130 P	30 S	44 S
5.05	101.8	102.3	101.8	102.0	88	132	108	112	32 D	86 D	32	160 P
5.30	102.0	102.2	102.0	101.6	100	120		100	32 V	160 P	40	
A. M.												
8.30	101.5	101.4	101.3	101.6	100	144	100	112	32	32	32	34

To determine the general action of the drug, four dogs in normal condition were given large doses of ethyl salicylate per stomach by means of a catheter. Table II contains the details of the experiments. In this table, V indicates vomiting, which in each instance was more of a regurgitation, apparently brought about by the irritation of the vapors arising in the throat, rather than typical emesis with retching produced by stimulation of the vomiting centre. D indicates drowsiness; S indicates sleep; P indicates panting with tongue out, seemingly due to an effort to expel vapors from the lungs rather than from absorption. The dogs are designated by the numerals 1,

2, 3, and 4. N indicates normal. While there are a good many irregularities shown in the details of the experiments, we may draw with considerable exactness the following conclusions:

a. The temperature gradually rises (minimum rise is 0.4° F. and maximum rise is 1.5° F.) during the first two hours after the drug is administered, then falls less quickly to normal.

b. The pulse-rate during first hour after giving drug in one instance was decreased, such slowing being probably due to vagus stimulation; in two of the animals it remained stationary, while in the other the normal pulse-rate was too irregular to draw any conclusion. All the animals show an increase in the pulse-rate during the next two hours (minimum increase four per minute, maximum thirty-two per minute). This increase in pulse-rate would seem to be due to the general excitement of the animal, as at about the same time there was also vomiting and panting.

c. The respiration during the first hour in all cases is slowed, probably due to stimulation of the terminations of sensory nerves of nose and mouth by the vapors of the drug. Soon, in each instance, the respiration becomes more rapid and the animal pants for a few moments, due to stimulation of respiratory centre.

d. In no instance did an animal show evidence of pain, nor was there any pronounced retching at the time emesis occurred.

e. All the animals vomited within two hours from the time the drug was administered, which was followed shortly by panting. It seemed to me that both depended upon the irritating effect of the vapors of the drug coming in contact with the mucous membrane of the pharynx and respiratory tract.

Administration of the same sized doses of the methyl salicylate to the same dogs at another time produced similar results, except that vomiting occurred sooner, accompanied by considerable retching and general uneasiness.

Several dogs were anæsthetized with chloretone and morphine to determine the effect of intravenous injections upon the blood-pressure, heart and respiration, the results being recorded on a kymograph. Reference to the tracings which are typical of a number taken gives the details of the experiments. In each instance an emulsion (ethyl salicylate, 10 per cent; alcohol, 50 per cent; water, 40 per cent) was injected into the femoral vein; a preliminary control injection of the 50 per cent alcohol was administered.

Experiment No. 4 may be taken as an illustration.

a. Experiment No 4. Dec. 6, 1904. — Dog. Weight, 12.5 kg. Preliminary treatment: chloretone + morphine. Cannula tied in right carotid. Blood-pressure recorded on kymograph. Electric respiratory recording apparatus. See Fig. 1.

TABLE III (Exp. No. 4).

Fig. 1.	Time.	Respiration-rate.	Blood-pressure. Height in mm. Hg.	Remarks.
	min.			
0	0.0	23	24	Normal.
1	2.0	23	19 Momentary	5 c.c. 50 per cent alcohol injected into femoral vein.
2	5.0	25 Soon becoming normal		5 c.c. mixture (ethyl salicylate 10 per cent + 50 per cent alcohol) femoral vein.
3	14.0			10 c.c. mixture.
4	15.0	Slow and irregular	Falls.	
5	16.5	Respiration ceases	Falls to zero and heart ceases to beat one minute later.	

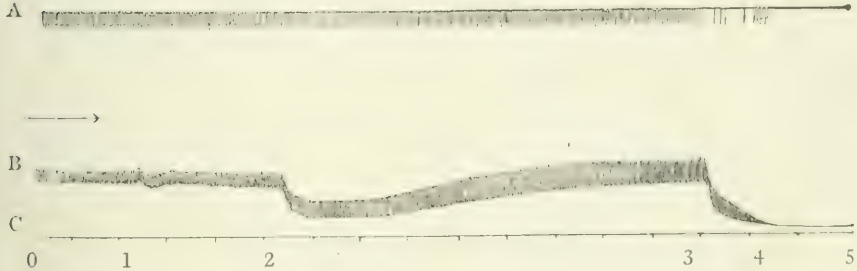


Figure 1. — Experiment 4. Explanation: The upper tracing, *A*, is the respiratory tracing; the middle tracing, *B*, is the blood-pressure tracing, and the lower tracing, *C*, is the time recorded in minutes. All the tracings were made simultaneously, and should be read from left to right. The numerals in the first column, Table III, and the numerals just below the blood-pressure tracing in Figure 1, which apply to all the tracings, correspond. For details, see Table III, reading horizontally across the page from the numerals in the first column.

In each instance the injections of the drug, if the dose were not too large, produced preliminary quickening of the respiration for a few minutes. Where a large dose was given, there was slowing, followed quickly by cessation of respiratory movements, and death from respiratory paralysis. At the same time the blood-pressure falls rapidly, and the heart is slowed. If the dose is not too large, the circulation

becomes normal in a few moments. It seems to me that the circulatory changes depend upon a transient stimulation of the vagus-centre accompanied by a direct paralyzing action upon the heart-muscle.

b. Experiment No. 8. Dec. 6, 1904. — Dog. Weight, 7 kg. Preliminary treatment: chloretone + morphine. Myocardiogram taken from right ventricle. Blood-pressure taken from right carotid. Artificial respiration.

TABLE IV (Exp. No. 8).

Fig. 2.	Time.	Heart.		Blood-pressure. Height in mm. Hg.	Remarks.
		Rate.	Amp. in mm.		
	min.				
1	0	112	45	28	Normal.
	5	110	38 Transient, tending to go into diastole	15 Transient	5 c.c. 50 per cent alcohol injected into femoral vein.
2	7	80	17 Transient, tending to go into diastole	Almost zero	5 c.c. of ethyl salicylate mixture injected into femoral vein.
3	10	128	56 Perfect heart-beats	21	5 c.c of ethyl salicylate mixture injected into femoral vein.
4	16	102	15 Systole very imperfect, heart greatly distended	Almost zero	
5	22	102	45	5	10 c.c. 50 per cent ethyl salicylate mixture injected into femoral vein.

Systole at once becomes very imperfect, and the heart distends widely, and about one minute later ceases to beat in diastolic condition, although artificial respiration is kept up for some time longer. See tracing on page 338.

From this and other similar experiments we may conclude that ethyl salicylate, when thrown directly into the circulation, stimulates the vagus, producing slight slowing of the heart; but that it exerts a very powerful local action on the heart-muscle itself, resulting in complete paralysis when large doses are given.

Summary.

Ethyl salicylate is a transparent, colorless, volatile, slightly soluble, oily fluid, possessing a pleasant, characteristic odor and taste.

Cultural experiments show that it has no antiseptic action.

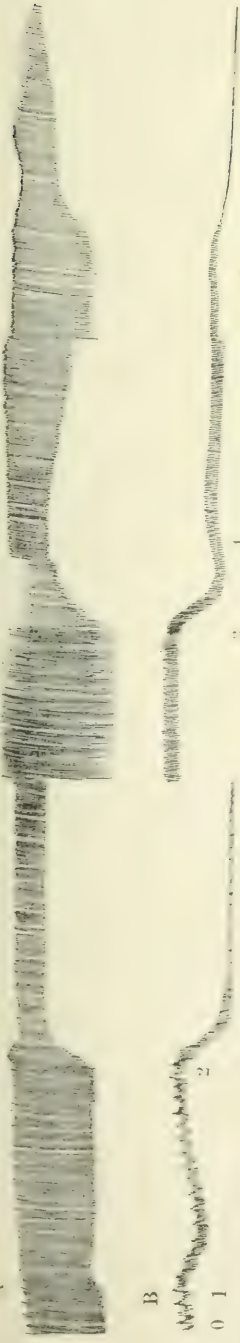


FIGURE 2. — Experiment 8. Explanation: The upper tracing, *A*, is a myocardiogram taken from the right ventricle, and the lower tracing, *B*, is the blood-pressure tracing taken from the carotid artery. Both tracings were made simultaneously, and should be read from left to right. The numerals in the first column of Table IV, page 337, and the numerals just below the lower tracing, which apply to both tracings, correspond. For details, see Table IV, reading horizontally across the page from the numerals in the first column.

Local action. — No irritation follows the application of ethyl salicylate to the skin or mucous membrane of the lips. It produces a slight burning sensation when applied to the more sensitive mucous membrane. Injected subcutaneously, the amount of irritation produced is slight, accompanied by a whitening of the tissues.

Toxicity. — Minimum fatal dose per gram body-weight for guinea-pigs is 0.0015 gm. internally, and 0.0014 gm. subcutaneously. Large doses given internally for several days in succession produce slight symptoms of gastric irritation, sufficient to produce vomiting, loss of appetite, finally death from inanition.

Absorption and excretion. — Ethyl salicylate is not absorbed from the respiratory tract, or when applied to the skin, but it is quickly absorbed from the stomach, or when given subcutaneously, and is rapidly excreted through the kidneys.

General action. — There is a gradual rise in temperature the first few hours after the administration of a single dose internally. The temperature then falls less quickly to normal. The pulse-rate is slowed at first, probably from vagus stimulation, later is accelerated on account of the general excitement of the animal. Respiration during first hour is slowed, but becomes rapid as the drug is absorbed probably from stimulation of the respiratory centre.

Blood-pressure, myocardiographic and respiratory experiments upon anæsthetized animals show that ethyl

salicylate stimulates centres in the medulla, producing, if the dose be large, increased frequency of respiration, lowering of blood-pressure, and a tendency for the heart to assume a diastolic condition. If the drug is pushed, the animal dies of paralysis of the respiratory centre, or, in case artificial respiration is supplied, the toxic action upon the heart is sufficient to produce diastolic standstill, for the most part due to the direct action of the drug upon the muscle itself.

In all cases the results obtained with ethyl salicylate were compared with those of the methyl compound, the latter being found much more toxic (minimum fatal dose 0.0007 per gram body-weight), produces more irritation of the alimentary canal, and is decidedly more irritating, evidenced by the appearance of the alimentary canal, when injected subcutaneously, etc.

THE CHEMISTRY OF MALIGNANT GROWTHS.— III. NUCLEO-HISTON AS A CONSTITUENT OF TUMORS.

By S. P. BEEBE.

[*Contributions from the Huntington Fund for Cancer Research, Department of Experimental Pathology, Cornell University Medical College, New York.*]

SINCE Kossel's[1] discovery of a peptone like body, histon, in the blood-cells of the goose, similar substances have been found to be important constituents of many tissues, notably the thymus gland and the testes of various fishes. These latter tissues have furnished the histon which has been most thoroughly studied, and in discussing the chemistry of this peculiar proteid the writer will refer to the results thereby obtained.

The characteristic properties of histons which differentiate them from other proteids are dependent on their strong basic character; they are precipitated by dilute alkalies, and are readily soluble in dilute acids. The precipitation by ammonia and by the alkaloid reagents in neutral solution are some of the most characteristic reactions of these substances. In the tissues the histons are associated with and very probably combined with nucleic acid; the combination thus formed being the so-called nucleo-histon, and since this combination appears to be similar to that between acids and bases to form salts, the term histonnucleate is coming into favor as being more exact. This combination is readily broken up; extraction with 0.8 per cent hydrochloric acid in the cold sufficing to split off all, or nearly all,[2] the histon and the insoluble nucleic acid (the prosthetic group of Kossel) is left on the paper after filtering. On the other hand, the nucleic acid combines readily with proteid in a slightly acid

[1] KOSSEL: Zeitschrift für physiologische Chemie, 1884, viii, p. 512.

[2] ACKERMAN, working under KOSSEL'S direction on the chemistry of blood corpuscles of birds, has found reason to believe that not all of the histon is split off by the use of 1 per cent hydrochloric acid. Zeitschrift für physiologische Chemie, 1905, xliii, p. 299.

medium to form a compound which appears to have the same properties as that obtained directly from the cells. There has been therefore a considerable doubt as to whether the nucleo-histon obtained by the usual method of precipitation from an aqueous extract of the pulverized tissue really was the nucleo-histon preformed in the cell nucleus, or whether the nucleic acid was to be found there mixed with proteid, and by the conditions of the precipitation a combination between the two was effected.

This question has been so thoroughly discussed by Bang,[1] Huiskamp,[2] Saint Hilaire,[3] Kossel,[4] Osborne,[5] and others that it is unnecessary to consider details here, and it suffices to state that the balance of opinion of these investigators is that the histon-nucleate occurs preformed in the cell and represents one fragment of its nucleus.

The older method of obtaining nucleo-histon from thymus extracts by precipitation with acetic acid as used by Lilienfeld and Kossel does not separate the nucleo-histon from the nucleo-proteid; as a consequence, the per cent of nucleo-histon in this gland as given by Lilienfeld is probably much too high. Many improvements and changes in the method have been made. Huiskamp and Bang[6] have used recently the method of precipitating the nucleo-histon by the use of calcium chloride in neutral solutions, and have found that a good separation of nucleo-histon from nucleo-proteid could be effected. The writer has used the method, and has found it to be quite satisfactory. After experimenting with various modifications, the following plan was adopted as being best suited to the conditions.

The tissue was thoroughly hashed, either in a meat grinder or by grinding with sand, the pulp mixed with several volumes of distilled water, and extracted in the cold for at least twenty-four hours, and in most cases for forty-eight hours, since nucleo-histon is not readily extracted. The extract was then filtered, first through cloth and finally paper, by the use of the suction pump. When the filter paper has been thoroughly plugged with the cell debris, the filtrate comes

[1] Bang: Beiträge zur chemischen Physiologie und Pathologie, 1904, iv, p. 351.

[2] Huiskamp: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 145.

[3] Saint Hilaire: Zeitschrift für physiologische Chemie, 1898, xxvi, p. 102.

[4] Kossel: Zeitschrift für physiologische Chemie, 1900, xxx, p. 520.

[5] Osborne: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 122; Report of the Connecticut Agriculture Experiment Station, 1901, p. 420.

[6] Bang: *Loc. cit.* p. 115.

through slowly but clear. In a number of cases reported in this paper the work of obtaining a clear filtrate has been very much simplified by the use of a powerful centrifuge. To this clear filtrate a sufficient quantity of a 10 per cent calcium chloride solution was added, to give a concentration of 0.1 per cent. If the extract contains nucleo-histon, a precipitate promptly forms, which is allowed to settle for twenty-four hours in a tall narrow jar, the supernatant fluid poured off, and the remaining precipitate centrifugated or filtered. Calcium chloride will precipitate some nucleo-proteid with the nucleo-histon, so that the first precipitate formed must be purified by dissolving in 0.2 per cent sodium chloride solution, filtering, dialyzing, and reprecipitating by the addition of calcium chloride to a concentration of 0.1 per cent. This process repeated twice gives a product free from nucleo-proteid. The nucleo-histon is now extracted with 0.8 per cent hydrochloric acid, the histon goes into solution, while the nucleic acid clumps together in a sticky mass. The presence of histon is demonstrated by neutralizing the acid filtrate and trying the well-known reactions with ammonia and the alkaloid reagents. In the filtrate from the first precipitation by calcium chloride the nucleo-proteid may be precipitated by the addition of acetic acid. A convenient test to determine when the nucleo-histon is free from nucleo-proteid is the Adamkiewicz reaction. Huiskamp[1] notes that nucleo-histon does not give this reaction, while nucleo-proteid does. The writer has tested the accuracy of this statement by trying the reaction on nucleo-histon prepared from calves' thymus by a variety of means, and has found it reliable.

In the course of his work on the chemistry of the lymphatic organs Bang[2] has examined a number of malignant growths for nucleo-histon by practically the same method as that given above. Five fibro-sarcomas from the breast, pharynx, and skin were examined with negative results. In no case could he obtain a precipitate in the water extract by the addition of calcium chloride. Acetic acid, however, gave a precipitate having the behavior of a nucleo-proteid. In the sixth case Bang examined a growth in the inguinal lymph gland, metastatic from a sarcoma of the testes extirpated about one year previously. Here he found a body having a phosphorus content of 5.18 per cent, and the reactions of the nucleo-histon normally found in lymph glands. Unfortunately the primary growth was not examined chemically, but Bang believes that the tissue which he used had its

[1] Huiskamp: *Loc. cit.* p. 164.

[2] Bang: *Loc. cit.* p. 362.

origin in cells directly transferred from the growth in the testis, and that chemically, therefore, the primary growth was similar to the metastatic growth in the lymph gland. The usual way of explaining metastases from malignant growths is to suppose that certain fragments of the primary tissue have been carried through the blood, or lymph tubes until stopped by some capillary circulation where, by continued division, a secondary growth, having an identical histological appearance with the primary, is produced. Hence the assumption that the secondary growth is chemically similar to the primary. Nucleo-histon is not a normal constituent of the mammalian testes, and reasoning from these premises Bang concludes that the primary growth had its origin in an embryonic, lymphatic cell group retained in the testes. The conclusion is an argument for Cohnheim's theory.

Following, in general, the method already outlined, the writer has tested a number of primary and secondary growths to determine whether nucleo-histon is a constant constituent of malignant tissues. Details follow.

1. Enlarged lymph gland from the axilla. The gland was about the size of a man's fist, and, as far as could be determined, was made up entirely of carcinoma tissue. Calcium chloride gave a heavy precipitate in the clear water extract. This precipitate dissolved partially in a 3 per cent sodium chloride solution. Dilution of the solution to a concentration of 0.9 per cent gave no precipitate, differing in this respect from extracts of the thymus gland. After dialyzing and adding calcium chloride to a concentration of 0.1 per cent a good precipitate was obtained. This precipitate was extracted with hydrochloric acid of 0.8 per cent strength. The filtrate from the gummy nucleic acid gave the ammonia precipitation test, and after portions of the filtrate were neutralized, the alkaloid reagents gave positive reactions. A small portion of the first precipitate obtained by calcium chloride was dried with alcohol and ether. It was found to contain 3.87 per cent phosphorus.
2. Four small carcinomas of the breast were ground together. Calcium chloride gave no precipitate in the clear filtered extract.
3. Epithelioma of the breast. Calcium chloride gave a small precipitate in the aqueous extract. This was dissolved in 2 per cent salt solution, and on dilution to 0.9 per cent sodium chloride, no precipitate formed. After dialyzing, calcium chloride caused an opalescence, but no precipitate.
4. Fibro-sarcoma from the thigh. Calcium chloride gave no precipitate in the filtered aqueous extract.
5. Melano-sarcoma from the tibia. Calcium chloride gave a precipitate which was redissolved and reprecipitated after dialysis. The final product gave

the *Adamkiewicz* and the Millon reactions. There was not enough material to try other reactions.

6. Scirrhus carcinoma of the breast. Calcium chloride gave a precipitate which seemed at first to be of some significance, but when it had been redissolved and reprecipitated twice, calcium chloride would then cause only an opalescence. A portion of the first precipitate gave the Adamkiewicz and Millon reactions very well, showing that it must have been made up largely of nucleo-proteid and not nucleo-histon.
7. Lymph gland and primary growth, carcinoma of the breast. The primary growth gave only an opalescence with calcium chloride. The lymph gland about the size of a hen's egg gave a bulky precipitate with calcium chloride; after this had been purified by reprecipitating twice, the final product was extracted with 0.8 per cent hydrochloric acid. The acid extract gave positive reactions for histon by precipitating with ammonia, and after neutralizing, the alkaloid reagents gave good reactions.
8. Carcinoma of the liver, metastatic from a primary growth in the stomach. The specimen was quite badly degenerated. Calcium chloride gave a small precipitate in the clear aqueous extract, but after solution in 3 per cent sodium chloride, and dialysis, calcium chloride gave only a slight opalescence. The growth was a large one, and a very large quantity of material was extracted by the water.
9. Sarcoma of the breast. Ground with sand and extracted with distilled water. Calcium chloride gave no precipitate, nor even an opalescence.
10. Two carcinomata of the breast. In the clear filtrate, calcium chloride gave only a slight opalescence.
11. Carcinoma of the liver, primary growth in the stomach. Calcium chloride gave only a slight opalescence in the clear filtrate of the aqueous extract.
12. Epithelioma of the cervix. Calcium chloride gave only an opalescence in the clear filtrate.
13. Round-celled sarcoma of the testes. The primary growth was badly degenerated, only a thin shell, 1 centimetre in thickness, being in a fresh condition. Calcium chloride caused only an opalescence.
14. Enlarged lymph gland in the axilla from a primary growth in the breast. The primary growth had been previously tested, and found not to contain nucleo-histon. The calcium chloride precipitate obtained from the enlarged lymph gland gave the characteristic reactions for nucleo-histon.

From the results of the detailed examination it is seen that the only undoubted cases in which nucleo-histon was found were the lymph glands. In Case 5, the melano-sarcoma, a precipitate was obtained which behaved like the nucleo-histon as far as solubility in sodium chloride solution, and precipitation by calcium chloride; but the final precipitate obtained was so very much smaller than the first as to

lead to the suspicion that the product was nucleo-proteid and not nucleo-histon. This suspicion was confirmed by the Adamkiewicz and the Millon reactions. The color reactions of histons obtained from various sources show many differences. The statements made by Huiskamp, which have been confirmed by the writer, referred only to the histon obtained from calves' thymus. Huiskamp did not quite understand how the histon could give a good Molisch reaction, and yet not respond to the Adamkiewicz test, since he supposed they were both dependent upon the presence of carbohydrate groups. We know now, however, from the work of Hopkins and Cole,[1] that the Adamkiewicz reaction is due to the tryptophan group. All true proteids give this reaction, but many proteid-like substances do not, gelatin, for instance. There are no statements in the literature regarding the color reactions of the histon obtained from the lymph glands of mammals, but from its close similarity to the thymus histon one is led to believe that it would not give the Adamkiewicz reaction.

Bang [2] has found the lymph gland nucleo-histon to be slightly different in its solubilities from that found in the thymus. On diluting the 2 per cent sodium chloride solution of thymus nucleo-histon to a content of 0.9 per cent sodium chloride, the nucleo-histon, according to Bang, is precipitated. The lymph gland nucleo-histon does not precipitate under similar conditions, and requires dialysis to remove the salt completely, and precipitation by calcium chloride. This precipitation behavior of the lymph gland histon was similar to that of the enlarged lymph gland containing the malignant cells. According to the writer's experience, the precipitation of thymus nucleo-histon under such circumstances is not invariable and is never complete. It depends on the concentration of the histon solution, the length of time it has been kept in solution, and the temperatures to which it has been subjected during its extraction.

Huiskamp states that sodium chloride, by a concentration of 0.9 per cent, will not completely precipitate the histon, and, furthermore, one requires fresh material. He says that if the extract from the glands has been kept for more then twenty-four hours, one obtains at best only a very small precipitate, and that the addition of chloroform to prevent putrefaction renders the precipitation by alkali salts difficult. My experience does not exactly agree with his in this respect. Chloroform works no harm if the extract be kept in the cold. As

[1] Hopkins and Cole: Journal of physiology, 1901, xxvii, p. 418.

[2] Bang: *Loc. cit.* p. 362.

usually employed, chloroform is added to prevent putrefaction when the extract cannot be kept in the cold. At ordinary room temperatures the extract is very soon injured, even if chloroform is present, so that it does not precipitate with 0.9 per cent sodium chloride, and only to a comparatively small degree with calcium chloride. This behavior is undoubtedly to be explained by the action of the enzymes which break down nucleo-proteid, and not by a direct action of the chloroform. The writer has obtained the best results by extraction in the cold for forty-eight hours.

It must be admitted that there are peculiar difficulties in dealing with nucleo-histon in tumors. It is practically impossible to get a tumor of any considerable size which is not degenerated to some extent, and nucleo-histon is easily changed by autolysis into a condition that does not precipitate with calcium chloride. In one of these tumors (Number 8) there was only a very small precipitate by the use of calcium chloride, and only a very small one (nucleo-proteid) by acetic acid. The tumor pulp suspended in distilled water had an acid reaction, which is most favorable for the autolytic enzymes breaking down nucleo-proteid. In the proteid free filtrate from this tumor, a substance having the properties of xanthin was precipitated by the alkaline silver method. Obviously the condition of the tumor was such that the method employed could not show whether nucleo-histon had been a constituent of the freshly growing tissue. In fact, no method could determine the point in such a tumor. This objection does not apply in most cases, and the enlarged lymph glands certainly autolyze as readily as other portions of the growth. They invariably yielded nucleo-histon. Many of the tumors used in these experiments were removed at operations, and were received at the laboratory in a perfectly fresh condition. The extraction was done in a cold room, and a few drops of chloroform were added to each jar of tumor pulp. The amount of nucleo-histon obtained from the lymph glands was much more than could be accounted for by the normal gland tissue which may have been present. It seems justifiable, therefore, to conclude from these results, and from those of Bang, that nucleo-histon is not a common constituent of primary malignant growths. Tumors primary in the thymus, however, would be expected to contain nucleo-histon. From its abundance in the secondary growths in the lymph glands, one is led to believe that, chemically at least, the metastases are not in all respects similar to the primary growth. The malignant cells have been influenced to

some extent by their environment, and although, histologically, the secondary growth is similar to the primary, there may be differences in chemical constitution.

A few observations on the chemistry of tumors that tend to confirm this view have been recorded in the literature. These are neither very complete nor satisfactory. The writer,[1] in the course of some work on the autolytic products of tumors, found glycocoll among the products of autolysis of a carcinoma from the broad ligament. In that paper it was remarked that "the tumor was growing on a structure, the broad ligament, which would be expected to yield collagen and consequently glycocoll. In this respect the tumor resembled the tissue upon which it was growing, or which had been eroded by its growth." This, however, was a primary growth and the conditions under which nucleo-histon was found in the secondary growths were somewhat different. It is to be expected that primary growths would be chemically similar to the normal tissue from which they started. In fact,[2] it seems that they may at times show a similar functional activity.

Neuberg[3] believes that he has found a reducing pentose as an autolytic product of carcinoma of the liver, but he did not find such a substance on the autolysis of the primary growth, carcinoma of the stomach. This interesting observation is not based on convincing experimental work. 300 gm. of the liver tumor were autolyzed five days, and Neuberg was able to isolate from the products 0.293 gm. of a reducing pentose. Only 15 gm. of the primary growth were autolyzed, and although no reducing product was found, a faint color reaction for pentose was given. After decomposing the carcinoma tissue by means of acid, Neuberg was able to recover nearly as much pentose from the stomach (1.68 per M) as from the liver (1.80 per M). Although the actual difference is small, the amount set free by autolysis is quite different. It is to be regretted that Neuberg did not confirm his results before hastening to publish them. It must be admitted that hydrolysis by means of acids does not always yield the same products as are obtained by the autolysis of the same tissue, yet this single observation can hardly be considered more than a suggestion. The writer believes, however, that Neuberg's ideas, as expressed in the following quotation, indicate an important field for

[1] Beebe: This journal, 1904, xi, p. 139.

[2] Schmidt: Virchow's Archiv, 1898, cxlviii, p. 119.

[3] Neuberg: Berliner klinische Wochenschrift, 1905, No. 5.

further study. "Bei Betrachtungen über chemische Vorgänge in carcinomatösen Organen genügt — sobald es sich um metastatische Gebilde handelt — nicht der Vergleich mit dem entsprechenden, normalen, Organen, sondern es ist ein solcher auch mit dem Primärgebilde notwendig, aus welchen die Zellen ausgewandert sind, resp. mit dem sie histologisch übereinstimmen."

FURTHER EVIDENCE OF THE FLUIDITY OF THE CONDUCTING SUBSTANCE IN NERVE.

A. J. CARLSON.

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EXPERIMENTS on the effects of stretching the nerve on the rate of propagation of the nervous impulse, using the pedal nerves and the foot musculature of the slug (Ariolimax), brought out the fact that stretching the nerve within certain limits increased the time required for the nervous impulse to travel between two fixed points of the nerve.[1] This increased time for transmission of the impulse is due to the increased length of the nerve in the stretched condition. The stretching of the nerve neither diminishes nor augments the rate of conduction as compared to that in the nerve which is not stretched, for when the rate is calculated from the transmission time and the actual length of the nerve in the two conditions, it is practically the same. This stretching of the nerve must not exceed the physiological limit, that is, the degree of extension of the nerve when the slug attains its greatest elongation in the act of locomotion. That the intensity of the nervous impulse is not altered by this degree of stretching of the nerve is inferred from the uniformity in the degree of contraction of the foot musculature. These facts tend strongly to support the view that the conducting substance in these nerves is in a fluid state, in consequence of which the stretching of the nerves merely extends the substance without altering the molecular state by stress or tension.

Similar results ought to be obtained in other nervous mechanisms which under normal physiological conditions are subjected to considerable variations in tension, such as the intrinsic cardiac nerve-plexus and the nervous tissue in the walls of the stomach. The nerve-cord of most annelid worms is another nervous mechanism normally subjected to great variations in extension and relaxation. The purpose

[1] JENKINS and CARLSON: Journal of comparative neurology, 1904, xiv, p. 85.

of this note is to record a series of observations on the influence on conduction of the stretching of the nerve-cord in these animals.

The method used in these experiments is a slight modification of that employed for measuring the rate of propagation of the impulse in worms and myriapods.[1] For the purpose of those measurements it sufficed to determine the length of the nerve-cord at the end of a series of experiments. During the experiments the worm preparation was always in loops to prevent it from breaking in two when being stimulated. For the present work, a simple contrivance was made by the aid of which the worm preparation could be put on

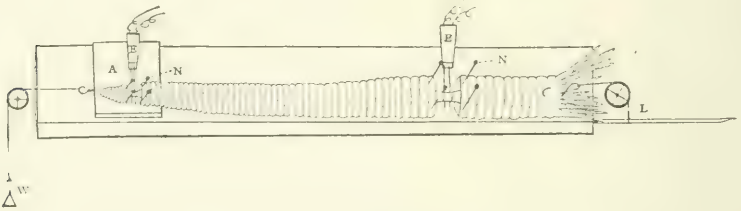


FIGURE 1. Diagram to illustrate the preparation of the worm for studying the effects of stretching on the conduction of the nervous impulse by the nerve cord.

A, cork platform, to which posterior end of worm was secured. This platform also carried the distal electrodes.

E, electrodes.

L, recording lever.

N, needles securing the worm to the platforms.

W, weights acting over friction wheel to stretch the worm.

stretch when it was being stimulated, and at the same time prevented from breaking in two when contracting. This device is represented in the diagram in Fig. 1. Instead of fixing the distal end of the worm preparation to the floor of the moist-chamber in the usual manner, it was secured to a small cork platform (*A*) which slid on friction wheels on the board. The distal electrodes were fastened to this platform. As the worm relaxed, the platform was moved back by means of a weight and string acting over a friction wheel. As the method of fixing the preparation to the board for the experiments prevented any stretching of the parts at the points of application of the distal and the proximal electrodes, any constant variation in the latent time of the preparation in the stretched and the relaxed condition must be ascribed to this difference in the condition of the por-

[1] JENKINS and CARLSON: Journal of comparative neurology, 1903, xiii, p. 259; CARLSON: Journal of experimental zoölogy, 1904, i. p. 269.

tion of the conducting path between the two pairs of electrodes, provided the rapidity and amplitude of the contraction of the reacting portion of the worm remain constant.

A number of different species of annelids were tried in these experiments, but the only species that proved serviceable was the large tube-inhabiting worm Bispira polymorpha (one of the Sabellidæ). In the case of the other annelids tried, either the latent time of contraction on stimulating the nerve-cord at different points was too variable, or else the stretching acted as a stimulus, keeping the preparation in constant motion. The large free swimming annelid Glycera rugosa reacts with but slight variations in the latent period on stimulation of the nerve-cord with single induced shocks, but when this worm is being gently stretched it begins to contract its longitudinal musculature even before the stretching has reached the maximal normal extension of the preparation.

The large tube-inhabiting worm Bispira reacts to all stimuli by quickly retracting the head end into the tube, or, in case it has been removed from the tube, by contraction of the longitudinal musculature of the anterior third of the body. A weak induction shock applied to the nerve-cord in any region of the body calls forth this reaction. Gentle stretching of the worm, even beyond the physiological limit, usually does not act as a stimulus.

Only the largest available specimens were used. When the worm is taken out of the tube and prepared for the experiments, it contracts until the distance of worm between the distal and proximal pairs of electrodes measures about 9 cm. or 10 cm. This maximal degree of contraction is not long maintained, the relaxation of the posterior third of the worm beginning within a minute or two. The amount of relaxation increases with the increasing fatigue of the preparation. The preparation of this worm does not extend uniformly when stretched, especially if it is in good condition. The relaxation and the stretching are greatest at the posterior end of the body, where the longitudinal musculature is less developed and becomes less anteriorly with increase in the development of the longitudinal muscles. The anterior fourth of the preparation was thus never stretched beyond the normal physiological limit, while the posterior third was in some cases, towards the end of a series of experiments, extended almost twice that of the normal.

It is evident from the diagram in Fig. 1 that stretching the worm, by pulling back the cork platform to which the posterior end of the

worm is secured, does not produce any variation in tension on the nerve-cord and tissues at the point of application of the proximal electrodes, or anterior to this point. The stretching of the worm behind the region of the proximal electrodes in no wise affected the latent period of the contraction of the head end on stimulation of the nerve-cord by the near electrodes. A few records on near stimulation thus sufficed to give data for calculating the actual transmission time between the two points stimulated. The latent period

TABLE I.

Detailed record of Experiment No. 1, Table II. Total latent time in seconds on stimulation of the nerve-cord by the distal electrodes.

	Worm stretched.	Worm relaxed.
	0.065	0.055
	0.065	0.055
	0.070	0.059
	0.060	0.056
	0.062	0.055
	0.066	0.055
	0.055	0.055
	0.070	0.056
	0.069	0.059
	0.074	0.057
	0.066	0.054
Average	0.066″	0.056″

Latent period on proximal stimulation: 0.021″.
Average length of the preparation in the stretched condition: 26 cm.
Length of the preparation after being killed in fresh water: 20 cm.
Rate in the stretched nerve-cord: 577 cm. per second.
Rate in the relaxed nerve-cord: 560 cm. per second.

on proximal stimulation being the same, the variation in the latent time on distal stimulation, according as the preparation is stretched or relaxed, thus constitutes an index of the effect of the stretching on conduction.

The uniformity of the reactions of the worm preparation in the stretched and the relaxed conditions appears from the measurements of the records of one series of experiments given in detail in Table I. These figures are typical of all the series. *The latent time is invariably greater in the stretched than in the relaxed condition of the worm, although the amplitude of the muscular response remains the same.* Unless the extension of the worm amounted to actual stretching, at least of the posterior end, this difference in the latent time did not appear. In no instance did the stretching diminish the latent time.

The close similarity in rapidity and strength of contraction of the head end of the preparation in the two conditions is shown by the tracings reproduced in Fig. 2.

It must be admitted, however, that the question whether the increased latent time is exactly counterbalanced by the additional length of the nerve-cord, so that the rate of conduction of the impulse remains exactly the same, cannot be answered with absolute certainty, because of the difficulty of obtaining accurate measurements of the length of the nerve-cord in the relaxed worm. The length of the nerve-cord in the relaxed worm is probably not far

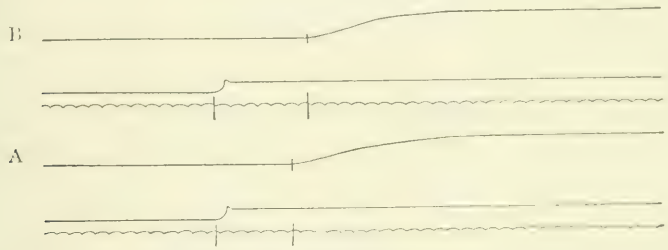


FIGURE 2. Tracings from the contraction of the head end of the worm (Bispira) on stimulation of the nerve-cord at the posterior end with a single induced shock. *A*, worm relaxed. *B*, worm stretched. Time: 100 d. v. per second. Difference in latent period between *A* and *B*, 0.01 second.

from the length of the worm after being killed in fresh water. At any rate, the nerve-cord does not appear to be in loops in the worm thus killed. Taking the length of the worm after being killed in this manner as the actual length of the nerve-cord in the relaxed condition of the preparation, the rate of conduction of the impulse in the stretched and the relaxed series in Table I is nearly the same. Some of the series in Table II show greater variations, probably owing to faulty measurement of the length of the cord involved. The average rate for all the series in Table II gives 630 cm. per second for the stretched nerve-cord, and 585 cm. per second for the relaxed nerve-cord. The slightly lower figure for the nerve-cord in the relaxed condition I am inclined to attribute to underestimating the actual length of the cord. The measurements of the length of the stretched cord are nearly accurate.

So far, then, these results in the worms support the same conclusion as the measurements on the pedal nerves of the slug. The stretching of the nerve-cord or the nerve produces actual extension

of the conducting substance without altering its molecular condition. These results do not point to any specific substance in the axis cylinder as being concerned in the conduction of the impulse, except that in case the neuroplasm is of a more fluid consistency than the neurofibrillæ they speak in favor of the former as the substance involved. This is contrary to the view advocated by Bethe. Accord-

TABLE II.

Summary of experiments on the effect of stretching the nerve-cord of worms on the rate of propagation of the nervous impulse. Measurements made on the polychæte worm Bispira polymorpha.

No. of experiment.	No. of records taken.		Mean latent time in seconds.		Length of cord in cm.	
	Stretched.	Relaxed.	Stretched.	Relaxed.	Stretched.	Killed in fresh water.
1	11	11	0.066	0.056	26	20
2	2	4	0.092	0.076	33	25
3	11	7	0.067	0.061	28	20
4	17	14	0.072	0.063	25	20
5	42	36	0.076	0.068	26	18
6	12	13	0.058	0.054	22	15
7	14	12	0.060	0.053	24	18
8	9	7	0.066	0.063	27	21
9	8	6	0.054	0.051	22	18

ing to Bethe, the neuroplasm is wanting at the nodes of Ranvier, and cannot, therefore, be the conducting substance in the nerve-fibre. This skilful investigator claims to have interrupted completely the neuroplasm of the axis cylinder by compression, leaving only the fibrillæ intact, and in that condition the axis cylinder still conducted the nervous impulse.[1] One must admit, however, that such experiments are difficult to perform, and the conclusions are open to the objection that in the fibres which retained their conductivity after the compression, the neuroplasm had not been completely separated, as slight amounts of it might escape detection by the microscope.

[1] BETHE: Anatomie und Physiologie des Nervensystems, Leipzig, 1903, p. 50.

As was stated, so far as could be determined from the muscular response, the stretching of the nerve-cord did not decrease or augment the intensity of the impulse. But this question needs further investigation by recording the electromotive force of the action current, for if the stretching actually diminishes the cross-sectional area of the conducting substance, that in itself would seem to imply a diminution of the energy of the nervous impulse at every point of the axis cylinder.

THE PHYSIOLOGICAL BEHAVIOR OF METHYLENE BLUE AND METHYLENE AZURE: A CONTRIBUTION TO THE STUDY OF THE OXIDATION AND REDUCTION PROCESSES IN THE ANIMAL ORGANISM.

By FRANK P. UNDERHILL[1] and OLIVER E. CLOSSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE uses to which methylene blue has been put are manifold. Therapeutically, it has been employed for years as a bactericidal agent, and as a stain for tissues and bacteria it is one of the best reagents known. Because of its property of readily undergoing reduction it has proved an excellent agent for the study of the reduction processes of the animal organism. Its behavior in the body and its elimination have been the subject of many investigations, the more important of which have been carried out by Ehrlich,[2] Dreser,[3] Müller,[4] Elsner,[5] and others,[6] who have shown that, when introduced into the body, methylene blue is excreted in the urine and fæces in the form of methylene blue, its leuco compound, and one or more chromogenic substances.

Recently, Herter,[7] in a study of the reduction processes of the organism by means of methylene blue, observed the appearance in the liver of a substance eliminated in the urine and bile which, upon treatment with acid, heat, and an oxidizing agent, was transformed from a colorless to a blue compound. From the nature of this sub-

[1] Research Fellow of the Rockefeller Institute for Medical Research.

[2] Ehrlich: Centralblatt für die medicinischen Wissenschaften, 1885, xxiii, pp. 113 and 163.

[3] Dreser: Zeitschrift für Biologie, 1885, xxi, p. 41; *Ibid.*, 1886, xxii, p. 56.

[4] Müller: Deutsches Archiv für klinische Medicin, 1899, lxiii, p. 130.

[5] Elsner: *Ibid.*, 1901, lxix, p. 47.

[6] Achard and Castaigne: Comptes rendus de la société de biologie, 1897, xlix, p. 1091; Brauer: Zeitschrift für physiologische Chemie, 1903–04, xl, p. 182; Herter: *Ibid.*, 1904, xlii, p. 403; also This journal, 1904, xii, pp. 128 and 207.

[7] Herter: *Loc. cit.*

stance, Herter was led to the opinion that methylene blue undergoes reduction to the leuco compound, which is in part excreted as a conjugate body, possibly a glycuronic acid compound. At the suggestion of Professor Herter, the nature of this "paired body" has been made the subject of the present investigation.

With What Does Methylene Blue Conjugate?

Various investigators have shown that when dilute solutions of methylene blue are slowly injected into the circulation of a rabbit, the dye is reduced to the leuco compound. The tissues appear normal when first exposed to the air, but soon undergo a change whereby they assume a blue-green color; or, if an oxidizing agent, such as hydrogen peroxide, is applied to the tissues, the change takes place immediately, the leuco compound being oxidized to the blue salt. According to Herter, it is possible to regain only part of the introduced dye in this way. In the liver, bile, and urine there is found a water-soluble compound, the "paired body," which does not become blue spontaneously or on boiling in the presence of an oxidizing agent, but which upon heating with an acid, like hydrochloric or acetic, grows intensely blue.

Preliminary experiments with rabbits which had received injections of methylene blue under conditions in every way comparable with those in Herter's investigation, yielded results in apparent agreement with them. For instance, after the slow injection of Grübler's methylene blue, the urine and bile, on boiling with acid and subsequent oxidation with hydrogen peroxide, assumed a deeper blue coloration than on oxidation alone. The liver extract behaved in a similar manner. Among the substances that most commonly unite with foreign compounds when introduced into the organism, are glycuronic and sulphuric acids, glycocoll and cholic acid. In order to show the presence or absence of a conjugate body formed by one of these compounds and methylene blue, the urine of a dog of 6 kgm. that had received an intraperitoneal injection of 50 c.c. of a 1 per cent solution of Grübler's methylene blue was studied. Search was made for conjugated glycuronates and glycocoll compounds with negative results. Three days after the injection of the methylene blue, the greater portion of the dye had been excreted, so that the urine for the two days following was of nearly normal color. In such a urine, color changes can be noted very readily. A study of these urines brought out the

following facts: acidification of the urine caused no change of color, but upon boiling an acidified urine the appearance of a pronounced green color was noted. If the urine was boiled for a period of three to five minutes without addition of acid, a result in every way similar was obtained. On treatment of the urine with an oxidizing agent (hydrogen peroxide), whether in acid or neutral solution, there was no immediate change of color; but when the solution was allowed to stand for a period of two hours, a green color was observed which was in every way comparable with that caused by boiling an acidified urine. If the urine, as excreted, stood in contact with the air for two hours, a decided green color developed.

From the foregoing observations it would seem that if a "paired body" is present in the urine after methylene blue injections, the conjugation cannot be of a very stable nature, inasmuch as the combination can be broken up by boiling. Such substances as the conjugated sulphates, glycuronates, etc., require far more vigorous treatment in order to effect the cleavage into the constituent bodies.

If the tissues (liver, muscles, and kidneys) of a rabbit that has received an injection of methylene blue (0.33 per cent solution) are ground with sand and repeatedly extracted with warm water a point can be reached after several days when the tissues become free from foreign coloring matter. Boiling the extracted tissues or their respective extracts in an acid solution produces no deepening of color. The extraction of the dye from the tissues is much more rapid when a faintly acid solution is employed than when water is used. The acid seems also to intensify the color of the extracts. So far as the tissues are concerned it would appear that if any conjugation with methylene blue does occur, it is in all probability of the nature of a combination between the cell protoplasm and methylene blue similar to that found by Mathews[1] and Heidenhain[2] for many dyes. According to these investigators, the proteids may be regarded as possessing both acid and basic properties. If a dye, like methylene blue, which is a salt, is added to an alkaline proteid solution, the base of the dye is set free and a union effected with the proteid. It can be readily seen how such a combination may be broken up by simple boiling, and why acid might be a much better solvent for the dye than water.

In view of the foregoing facts, the conclusion seems warranted that

[1] Mathews: This journal, 1898, i, p. 445.

[2] Heidenhain: Archiv für die gesammte Physiologie, 1902, lxc, p. 113, and 1903, lxcvi, p. 440.

the evidence of a "paired body"[1] in the organism after injections of methylene blue is insufficient.

The Excretion of Methylene Blue.

In the course of the observations on dog's urine excreted after the injection of methylene blue, it was noted that if such a urine is made faintly ammoniacal and extracted with ether (or chloroform), the ether assumed a beautiful red color, while the urine regained its normal appearance. The addition of a few drops of acid to the ethereal solution caused the formation of two layers of liquid, the upper (ethereal) becoming colorless, while the lower turned an intense blue or blue-green. Again making the ethereal solution ammoniacal, and shaking, led to the reappearance of the red color in the ether, and to the decolorization of the aqueous layer. Distillation of the ether from the red ethereal solution resulted in a blue residue, regardless of the reaction of the fluid. An examination of this coloring matter showed that it presented an absorption spectrum differing slightly from that given by methylene blue. In ethereal solution it yields no absorption bands. Similar results could be obtained with aqueous extracts of the several tissues of animals that had received methylene blue injections. A large quantity of the same substance could be demonstrated in the commercial methylene blue employed for the injections.

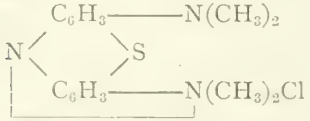
A study of the chemistry[2] of methylene blue has shown that the methylene blue preparations available (Grübler's, Kahlbaum's, crystalline, and Merck's, medicinal) are mixtures of methylene blue and methylene azure. Methylene azure is formed from methylene blue by treatment with strong alkalies, and is a contamination which it is almost impossible to separate from methylene blue. Like the latter, it may exist in three forms: (*a*) the free base, (*b*) a salt, (*c*) the leuco compound. The latter is formed by reducing agents with as great ease as the leuco methylene blue is formed from its precursor. The free base of methylene blue is blue and insoluble in ether. The free base of methylene azure is red and soluble in ether and chloroform. It has a great affinity for acid, since simply pouring an acid

[1] The idea of a conjugation after methylene blue injections is by no means new. Dreser (*Loc. cit.*) put forth the same view in 1885, but it could not be confirmed by Ehrlich (*Loc. cit.*).

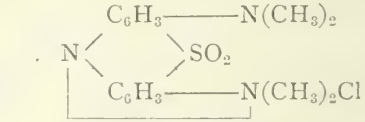
[2] For the chemistry of methylene blue, see Bernthsen: Liebig's Annalen der Chemie, 1885, ccxxx, p. 73; and 1899, ccli, p. 1.

into an ethereal solution will render the ether colorless, while the acid becomes blue, owing to the formation of a salt which is blue. This blue salt has properties in every way identical with methylene blue, with the single exception of its spectrum. In strong solutions, methylene blue gives a spectrum with two absorption bands, the broader one, between *B* and *C*, the other between *C* and *D*. In dilute solutions, only the more intense band may show. In strong solutions, methylene azure also has two absorption bands, but both are between *C* and *D*. Only one band appears in dilute solutions. The salts of both methylene blue and methylene azure are insoluble in ether. The salt of methylene blue is slightly soluble in chloroform, while the leuco compounds of both dyes are insoluble in alkalies and water.

That methylene azure is a contamination originally present, and is not formed from methylene blue by the slight addition of ammonia, may be seen from the following experiment. Methylene blue (Kahlbaum, crystalline) was dissolved in water, made faintly ammoniacal, and shaken with ether until the ether was no longer tinged with red. The purified aqueous solution was then made distinctly ammoniacal, and allowed to stand for one hour. At the end of this time no methylene azure could be detected. After a period of twelve hours, however, a noticeable quantity of methylene azure had been formed. While several volumes of concentrated ammonia are necessary to rapidly convert a portion of the methylene blue into the azure compound, dilute ammonia will tend, on long standing, to accomplish the same action.[1] The quantity shaken out by a faintly ammoniacal ethereal solution cannot, however, be accounted for by the action of the ammonia upon the methylene blue. Addition of ammonia, in the quantities employed here, transforms the methylene azure from the salt to its free base form. Methylene azure differs in composition from methylene blue only by containing two atoms of oxygen. Thus:



Methylene blue(salt)



Methylene azure (salt)

The transformation of methylene blue to methylene azure appears to be very easily effected. It is unknown whether the reverse change can take place.

[1] *Cf.* BERNTHSEN: *Loc. cit.*, 1885, p. 169.

In order to study the behavior of methylene blue in the body, it is obviously desirable that the dye be in a form uncontaminated. Accordingly, experiments have been carried out with this purpose in view. The results show that the only satisfactory method of purification which gives a methylene blue solution free from methylene azure is the following: solutions of methylene blue are made distinctly ammoniacal and shaken with ether until the latter is no longer colored red. The process is long (one to two days, according to the strength of the solution) and requires large volumes of ether. After the methylene azure has been removed, the methylene blue solution is made acid with hydrochloric acid, and concentrated to a smaller volume. On cooling, the methylene blue salt crystallizes in long needles. Acid solutions of methylene blue remain unchanged for indefinite periods of time, but alkaline solutions, on standing for some hours, readily give evidences of the presence of methylene azure, and after three weeks such solutions yield large quantities of the azure compound.

The behavior of pure methylene blue obtained by the method outlined above has been studied in the organism of man, dog, cat, and rabbit. Typical protocols follow:

Experiment 3. — A rabbit of 3 kgm. under ether anæsthesia, received an intravenous injection of 87 c.c. of a 0.5 per cent solution of *pure methylene blue* in 55 minutes. The tissues presented the appearance usual after methylene blue injection, that is, they rapidly changed from the normal color to a blue or blue-green hue. On addition of hydrogen peroxide or acetic acid, an intense blue coloration developed immediately. The urine and bile, when made ammoniacal and shaken with ether, gave evidence of the presence of both methylene blue and methylene azure. Only traces of methylene azure were noted in the urine; while in the bile, methylene azure could be shown in much greater quantity than methylene blue. The muscles, liver, stomach, and kidneys were ground finely and extracted with warm water until all foreign coloring matter had been taken out. All of the aqueous extracts were found to contain a little of the free methylene azure base. This was ascertained by shaking the extract with ether, whereupon the ether became tinged with red. A small quantity of methylene azure, and a much larger amount of methylene blue, was present. Boiling the extracted tissues and the aqueous extracts with acid did not cause a change of color.

Experiment 4. — 10 c.c. of a 0.35 per cent solution *pure methylene blue* were intravenously injected into a rabbit of 1200 gm. and under ether anæsthesia. Examination of the urine of the following day revealed the

presence of relatively large quantities of methylene azure and only traces of methylene blue. The second day following the injection, traces of methylene azure only could be found. The urine on the third day was normal.

Experiment 5. — To a cat of 2.5 kgm. were administered intravenously 10 c.c. of a 0.25 per cent solution of *pure methylene blue.* No urine was excreted until the third day after the injection. This urine was perfectly normal in appearance, and gave no evidence of chromogenic substances on oxidation, or on boiling after acidification.

Experiment 6. — A cat of 3 kgm. received an intravenous injection of 20 c.c. of a 0.35 per cent *pure methylene blue* solution. The next day no urine was excreted. On the second day the urine contained much methylene blue and significant quantities of methylene azure. Fæces passed on this day also contained traces of both substances.

Experiment 7. — A dog of 8.5 kgm. received an intraperitoneal injection of 20 c.c. of a 0.35 per cent solution of *pure methylene blue.* The urine of the next day contained both methylene blue and methylene azure.

In order to test the behavior of methylene blue in the organism of man, Merck's medicinal methylene blue was purified in the manner previously described. A typical experiment follows:

Experiment 8. — To three subjects, A, B, C, *pure methylene blue* was administered in capsules containing doses of 75, 50, and 75 mgm. respectively. One and one-half hours later the urines of all three subjects contained significant quantities of methylene azure and larger amounts of methylene blue. Two days later all the methylene blue had disappeared, but a trace of methylene azure could be shown. Both compounds were found in small amounts in the fæces.

A review of the experiments outlined above shows that pure methylene blue, whether administered intravenously (cat and rabbit), intraperitoneally (dog), or by way of the mouth (man), in relatively large doses is eliminated by the kidneys and intestines in the same forms, that is, as methylene blue and methylene azure. The presence of the latter body in the urine and fæces is unquestionably due to the oxidative changes going forward in the organism, whereby the methylene blue is in part oxidized to methylene azure. When pure methylene blue is injected intravenously in *small* doses (10 c.c. of a 0.25 per cent solution), in the cat at least, the dye, or its known decomposition products, fails to appear in the urine or fæces.

If the freshly voided urine of an animal that has received an injec-

tion of methylene blue is boiled for several minutes, the blue coloration is intensified, although another portion of the same urine does not show any deepening of color on treatment with an oxidizing agent. Acidification of the urine with subsequent boiling causes the blue coloration to deepen in degree equal to that caused by several minutes boiling without addition of acid. The depths of color in the two cases are not to be distinguished from one another. If the urine is vigorously and repeatedly extracted with ether, the color changes of the urine noted after boiling and acidification and boiling no longer appear. The ethereal extract remains colorless. When the latter is treated with acetic acid, there is a formation of two layers of liquid, the upper, ethereal layer remaining colorless, the lower, acid layer becoming deep blue. Made ammoniacal and shaken, the ethereal solution becomes red, while the alkaline fluid is blue. Spectroscopic examination of these two solutions after acidification shows that the red ethereal layer is a solution of methylene azure, and that the blue ammoniacal solution consists of methylene blue. From these results it would seem that methylene blue is excreted also in the forms of the leuco compounds of both methylene blue and methylene azure. Additional experiments bear out this conclusion. A portion of the colorless ethereal extract of an experimental urine treated with water, and vigorously shaken to give large surface exposure to the air, gradually assumed a deep blue color, which could be shown to consist of a mixture of methylene blue and methylene azure. Addition of water to a second portion, without shaking, did not cause a development of color on standing for one hour. From another portion, the ether was evaporated, and the blue residue, when taken up in water, was found to consist of a mixture of methylene blue and methylene azure. Addition of hydrogen peroxide to a fourth portion of the ethereal extract caused the formation of a blue solution.

From the experimental observations noted, the conclusion seems justified that when methylene blue is introduced into the animal organism, it is excreted in at least four forms: namely, methylene blue, methylene azure, and their corresponding leuco compounds. The two latter substances are not oxidized so readily as one would expect from a perusal of the literature on the subject, as may be seen from the following experiment. 1. A solution of pure methylene blue is completely decolorized by ammonium sulphide, and is divided into two portions. One portion very rapidly turns blue on exposure to the air, while the other assumes an intense blue color when treated

with hydrogen peroxide. 2. A pure methylene blue solution is completely decolorized with ammonium sulphide, and at once made acid with acetic acid, and then divided into five portions. The first portion is allowed to stand in a shallow dish exposed to the air. After a period of twenty-five to thirty minutes, the colorless solution begins to assume a blue coloration, which very gradually deepens. Hydrogen peroxide added to a second portion causes a development of color after fifteen to twenty minutes. Through the third portion a current of air is passed, to expel the hydrogen sulphide gas. The appearance of a blue color is not to be observed until fifteen to twenty minutes after the expulsion of all the hydrogen sulphide gas, and then the color development is extremely slow. If the fourth portion is boiled for one to two minutes, the solution becomes intensely blue. If to the fifth portion alkali is added until the reaction is alkaline, the blue color returns at once. Here it will be noted that the results found are identical with those that obtain in a methylene blue urine; for if such a urine is boiled for two to three minutes, it deepens in color. Addition of acid, with subsequent heating, causes the development of the blue color more rapidly. Standing in the air will cause the methylene blue urine to assume gradually a deeper hue, as will addition of hydrogen peroxide and standing for a certain length of time.

What is the explanation for the difference in behavior between an alkaline and an acid solution of the reduced methylene blue? Ordinary methylene blue is a salt. When ammonium sulphide is added to a methylene blue solution, two things happen: namely, the alkali transforms methylene blue (salt) into methylene blue (base), thus:



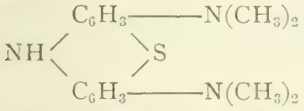
Methylene blue (salt) Methylene blue (base)

while the latter is reduced to the leuco compound, also a base, thus:

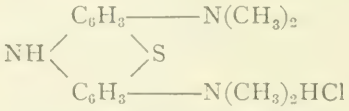


Methylene blue (base) Leuco methylene blue (base)

so that the solution contains the leuco compound of methylene blue which is a base. Standing in the air, or treatment with an oxidizing agent, causes this substance to be quickly changed to the colored compound — the methylene blue base. If, however, the solution of the leuco methylene blue base is made faintly acid, the oxidation does not ensue so readily.[1] A new compound, less easily oxidized, has been formed. Represented graphically, the change would be as follows:



Leuco methylene blue (base)



Leuco methylene blue (salt)

It is this leuco compound, the salt,[2] which is the substance probably present in the urine. It is the reaction of the medium containing the leuco body which is the essential factor; for when the solutions are alkaline, the oxidation of the leuco compound is rapid, whereas blue color develops very slowly in the presence of acid. Observations similar in every respect can be made with methylene azure. The weaker the acid medium, the more striking are the results that may be obtained. Acid potassium phosphate acts much better than acetic acid, and an acetic acid medium is much more favorable than one made acid with hydrochloric acid. Here the results obtained with a methylene blue solution are identical with those that may be observed in a methylene blue urine. The objection might be offered that when a colorless ammonium sulphide solution of the methylene blue compound is made acid, sufficient hydrogen sulphide is present to prevent the oxidation, that is, hydrogen sulphide will still function as a reducing agent, and hence the solution will remain colorless. Two observations speak against such an argument. In the first place, even though there is a large excess of hydrogen sulphide present in such a solution, addition of an alkali causes the immediate appearance of the intense blue coloration. Secondly, when the hydrogen sulphide is removed by a current of air, the solution does not become blue until many minutes have elapsed.

This behavior of methylene blue and its derivatives is sufficient to account for the reactions attributed by Herter to a "paired sub-

[1] *Cf.* Bernthsen: *Loc. cit.*, 1885, p. 149.

[2] Regarding the existence of such a compound, see Bernthsen: *Loc. cit.*, 1885, p. 150.

stance." The presence of the four substances, methylene blue methylene azure, leuco methylene blue (salt), and leuco methylene azure (salt), has been demonstrated in the urine of the dog, cat, rabbit, and man, after the introduction of pure methylene blue. In the aqueous extracts of the liver, muscles, kidneys, and bile of the rabbit, similar compounds may also be shown after injections of pure methylene blue.

A single experiment was performed to determine whether the methylene blue was absorbed into the lymphatic system. The protocol follows.

Experiment 9. — Lymph was collected from the thoracic and cervical ducts of a dog of 20 kgm. under morphine and A. C. E. anæsthesia. The urine was obtained from cannulas in the ureters, and the bile from a biliary fistula. 200 c.c. of crude methylene blue solution were injected into the facial vein of the animal in two hours and thirty minutes. One hour and twenty minutes after the beginning of the injection, the urine was colored blue, and twenty minutes later the bile contained the dye. The cervical lymph remained free from coloring matter, and in the lymph from the thoracic duct, the presence of chromogenic substance was doubtful. This lymph was somewhat tinged with blood, and yielded no more color on oxidation than might be expected from slight contamination with blood containing the leuco compounds. On oxidation, none of the organs, except the kidney, showed the least trace of methylene blue. Treatment of this tissue with an oxidizing agent transferred the intensity of the blue color from the medulla to the cortex. The blood of the animal had not clotted thirty-six hours after death.

It appears in this case at least that the methylene blue is transformed or excreted before it is distributed at all widely in the lymph spaces. Trials were also carried out to determine whether isolated surviving organs, such as the liver, kidney, and spleen are capable of transforming pure methylene blue to methylene azure. The results were negative.

The Excretion of Methylene Azure.

It has been shown above that methylene blue is in part transformed into methylene azure when introduced into the body. The behavior of the latter substance in the organism has been made the subject of further study.

Spectroscopic examination of a methylene azure preparation labelled

"Methylene azure hydrochloride, puriss,"[1] indicated the presence of a relatively large quantity of methylene blue. Accordingly, pure methylene azure was prepared from impure methylene blue in the following way. A strong solution of the dye was treated with strong ammonia, and extracted with ether in a Kutscher-Steudel apparatus for several days. The extract was freed from ether and ammonia by heating, and the residue extracted with water. The methylene azure in the aqueous solution was shaken out with ether, and the latter removed by distillation. A 0.05 per cent solution was used for the injections. Three typical experiments are appended.

Experiment 10. — Into a rabbit of 1200 gm. were injected intravenously 90 c.c. of a 0.05 per cent solution of *pure methylene azure* in fifty minutes. Twelve minutes later the animal was killed by bleeding. The blood serum contained a trace of methylene azure. The tissues appeared normal and did not reveal a trace of blue coloration after treatment with hydrogen peroxide or acetic acid. The bile contained a little methylene azure, while the urine was free from it. The only organ or tissue that gave evidence of foreign coloring matter was the stomach with its contents. Of the aqueous extracts of the liver, muscles, kidneys, small and large intestines, and stomach examined, only the liver and stomach revealed the presence of a dye; here only traces of methylene azure and its leuco compound.

Experiment 11. — A rabbit of 2.5 kgm. received an intravenous injection of 20 c.c. of a 0.15 per cent solution of *pure methylene azure* in fifteen minutes. After ten minutes the animal was killed. A trace of methylene azure was detected in the blood serum. The tissues appeared normal; but upon treatment with acetic acid or hydrogen peroxide, assumed a green coloration that was just discernible. A trace of leuco methylene azure and methylene azure could be demonstrated in an aqueous extract of the muscles. The urine contained methylene azure and leuco methylene azure (salt), and behaved toward acid and oxidizing agents in a manner similar to a methylene blue urine.

Experiment 12. — 30 c.c. of a 0.15 per cent solution of *pure methylene azure* were administered intraperitoneally to a dog of 10 kgm. The urine as well as the fæces of the following two days contained methylene azure and the leuco compound. Subsequently the urine contained no colored bodies. The total quantity recovered was decidedly smaller than that injected.

Experiment 13. — After the intravenous injection of 10 c.c. of the same solution of methylene azure into a cat of 3 kgm. no trace of the dye could be detected in the urine or fæces.

[1] Kindly furnished by Professor HERTER.

Such experiments demonstrate that when methylene azure is introduced intravenously (in the rabbit and cat) or intraperitoneally (in the dog) it reappears in the urine and fæces in part as the unchanged dye and partly as a leuco compound. Both forms are found in the tissues. Furthermore, when a small enough dose is given, none can be regained from the urine or fæces. Even after a large dose, the quantity excreted is far less than that introduced. The major portion does not reappear, at least in a chromogenic form. From this it may be concluded that the dye has undergone a change, probably oxidative in character, whereby the methylene azure is transformed into one or more decomposition products, the nature of which is at present unknown.

Elsner[1] has studied the excretion of methylene blue quantitatively, and has shown that at most only 50 to 60 per cent of the introduced dye can be regained in the urine and fæces. Müller[2] found that methylene blue is eliminated from the animal organism in at least four forms, namely, methylene blue, an unknown oxidation product of the dye, and two unknown chromogenic substances. The present confirmatory experiments afford an explanation of the results of both investigators. The unknown oxidation product of Müller is in all probability methylene azure, and the leuco compounds of the writers are probably identical with Müller's unknown chromogenic substances. The explanation of Elsner's results lies in the observation that methylene blue may in part be transformed to methylene azure, and that the latter is partly decomposed, and does not reappear as a chromogenic substance.

The results obtained *in vivo* may be imitated in test-tube experiments. Solutions of *pure methylene blue* heated with dextrose undergo no change. If a drop of potassium hydroxide is added to the hot solution, two reactions occur, — the solution is decolorized (leuco methylene blue (base) is formed and separates), and a significant quantity of methylene azure (in the leuco form) arises. Boiling the methylene blue solution in the presence of potassium hydroxide causes the formation of the methylene azure without the development of any reduction product. Ammonia substituted for potassium hydroxide does not lead to a formation of methylene azure, although the reduction takes place. Here the alkali seems to be the effective factor; possibly in the organism the transformation of methylene blue into

[1] Elsner: *Loc. cit.*

[2] Müller: *Loc. cit.*

its oxidation product, methylene azure, is facilitated or inaugurated by the presence in the tissues of traces of alkaline salts.[1] The products formed suggest that the processes involved are essentially those of oxidation and reduction, which may possibly occur simultaneously in the same tissue or tissues. Thus the fate of methylene blue demonstrates not only the reduction processes of the body, but also those of oxidation, and presumably the conditions that modify the former also exert an influence on the latter.

Summary.

The evidence offered of a conjugate body formed in the organism after the introduction of methylene blue is insufficient. The experimental facts can be accounted for by the presence in significant quantities of two chromogenic substances, leuco compounds corresponding to methylene blue and methylene azure.

The introduction of pure methylene blue, whether intravenously, intraperitoneally, or by way of the mouth, is followed by the appearance in the urine and fæces of methylene blue, methylene azure, — an oxidation product of methylene blue, — as well as leuco compounds corresponding to methylene blue and methylene azure.

When pure methylene azure is injected, intravenously or intraperitoneally, only a portion can be regained, consisting of methylene azure and a leuco compound of this dye. Small doses of either methylene blue or methylene azure fail to reappear in the urine. It is only when the oxidative processes of the organism are inadequate that these bodies are manifested. The present experiments therefore demonstrate the simultaneous action of both oxidation and reduction processes in the animal organism.

[1] If a solution of crude methylene blue is treated with calcium dioxide, or magnesium dioxide, and tartaric acid, the depth of color of the solution is markedly decreased. Examination shows that the greater portion of both methylene blue and methylene azure present has been oxidized to colorless substances. Hydrogen peroxide fails to call forth this reaction.

ON THE UNION OF A SPINAL NERVE WITH THE VAGUS NERVE.

By JOSEPH ERLANGER.

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Introduction.

IN a recently published article on nerve union, Langley and Anderson[1] have formulated the law that "the central end of any *efferent somatic*[2] fibre can make functional union with the peripheral end of any *preganglionic* fibre. . . ." But according to their review of the literature, it would appear that no conclusive evidence has as yet been obtained that motor fibres will make functional connection with the preganglionic fibres in the vagus nerve. Indeed, as late as 1900, Langley[3] stated that "a recovery of function, after section of the vagus nerve, has not been observed so far as regards its autonomic fibres. . . ."

But few attempts have been made to obtain union of efferent somatic fibres with the preganglionic fibres of the vagus nerve. Flourens[4] seems to have been the first investigator to have undertaken it. In 1828 he reported some experiments in which the fifth cervical nerve was sewn to the peripheral end of the vagus. He obtained no signs of functional union. In 1885 Rawa[5] sutured the hypoglossal to the vagus nerve in several species of animals. The physiological tests for regeneration were made from eighteen to twenty months after the operation and some days after cutting the vagus and hypoglossal nerves of the opposite side. He found that the sutured nerve behaved, so far as concerned its action on the heart, in every respect like a normal vagus nerve. Through it both direct and

[1] Langley and Anderson: Journal of physiology, 1904, xxxi, p. 365.
[2] Italicized words have been substituted for letters in the original.
[3] Langley: Schäfer's Text-book of physiology, London, 1900, ii, p. 665.
[4] Flourens: Ref., Langley and Anderson, *Loc. cit.*
[5] Rawa: Archiv für Physiologie, 1885, p. 296.

reflex inhibition of the heart were obtained, and through it the central nervous system exercised a tonic inhibitory control over the heart. But Langley and Anderson raise the valid objection to the work of Rawa that, in his final tests, cognizance had not been taken of the possibility of a downgrowth of fibres from the central stump of the divided vagus nerve. But although these experiments do not demonstrate conclusively that fibres of the hypoglossal nerve may usurp the functions of vagus fibres, still they prove that functional regeneration of the visceral fibres of the vagus nerve does occur.

The experiments of Henri and Calugareanu,[1] who cross-sutured the hypoglossal and vagus nerves, are open to the same objection, and their results have the same significance as Rawa's.

It was, therefore, to be expected that the negative results, reported by Langley and others, of experiments testing the power of the vagus to regenerate itself, would be accounted for by the brevity of the time allowed for regeneration. This was actually demonstrated in Langley's laboratory by Tuckett,[2] who found, in the rabbit, that three years after operation sutured vagi respond to stimulation almost normally.

While reviewing the literature on the subject of regeneration of the vagus nerve, we were impressed by the comparative sluggishness in the return of function after unions with it, a sluggishness which seems to be out of all proportion to the length of the nerve. At this time two possible explanations suggested themselves: (a) The small medullated fibres which occur in the vagus nerve, and which are probably its preganglionic fibres, may not grow as rapidly as large medullated fibres. (b) In the process of regeneration, fibres which are normally short may not extend beyond their normal length as rapidly as fibres whose normal length more nearly approximates that required of them in the regeneration.

We have no experimental evidence in support of the first possibility. Indeed Langley and Anderson[3] state, we think, however, without sufficient justification, "that small nerve fibres can much more readily unite with large ones than large ones with small."

With regard to the second possibility, if there is any truth in the

[1] Henri and Calugareanu: Comptes rendus de la société de biologie, 1900, lii, p. 503; Journal de la physiologie et de la pathologie générale, 1900, ii, p. 709.

[2] Tuckett: Journal of physiology, 1896, xix, p. 297. In this article negative results are reported. The completed work is reported in Journal of physiology 1900, xxv, p. 303.

[3] Langley and Anderson: *Loc. cit.*, p. 379.

statement made by Langley[1] that "the axon process of each cell wherever it is cut will, in favorable conditions, grow to its own length, neither more nor less," it is hardly to be expected that the short fibres of the hypoglossal could grow down to the heart. And the hypoglossal is the only nerve that has been used for this purpose in recent years. Although the latest work of Langley and Anderson[2] does not bear out tacitly the above-stated dictum, nevertheless we might be justified in expecting that the union of efferent spinal fibres with the vagus nerve would offer better chances of functional regeneration than the union of hypoglossal with vagus.

Furthermore we hoped that should signs of functional regeneration be obtained after the union of a spinal nerve with the vagus nerve, the interpretation of the results would not offer so many difficulties as in the case of union of one cranial nerve with another.

For these reasons it was decided to try, in the dog, the effect of uniting one of the branches of the cervical plexus with the peripheral end of the vagus nerve.

Methods.

With this object in view six dogs were prepared. In all excepting one the vagus nerve was cut at a point about 2 cm. above the inferior cervical ganglion, and the peripheral stump was sutured to the central stump of the most accessible branch of the brachial plexus. In most instances this was probably the trunk formed by the union of twigs from the fifth and sixth cervical nerves. In the case of the first dog operated upon, the central end of the hypoglossal nerve was united with the peripheral end of the vagus nerve. The cut surfaces of the nerves were secured to one another by two fine silk sutures passed on opposite sides of the nerve. No attempt was made to keep the sutures within the limits of the sheath of the nerves. In each instance, excepting the case of union of hypoglossus with vagus, a piece 4 to 5 cm. long was excised from the central stump of the vagus. The operations were performed aseptically, and none of the wounds became infected. Morphine and ether were used as anæsthetics. At the preliminary operations, the ether was administered through a cone; at the final operations, it was administered through a tracheal cannula.

[1] Langley: Journal of physiology, 1900, xxv, p. 417.

[2] Langley and Anderson: *Loc. cit.*

In order to test whether or not regeneration had occurred, a second and sometimes a third operation was performed, at which the sutured nerve was exposed and stimulated. The effect of this upon the pulse-rate was determined graphically with the aid of the sphygmomanometer devised by the author.[1]

In each experiment the nerves were examined histologically. As a rule cross-sections of the nerves were stained by the methods of van Giesen and of Weigert.

The experiments will be presented in the order in which the final tests for regeneration were made.

Experiments.

Dog No. 2. *Union of the peripheral end of the right vagus nerve with a branch of the right cervical plexus.* — Operation Feb. 26, 1904. At the second operation, performed 80 days later (May 16), it was found that the two nerves had united beautifully. The central end of the vagus terminated in a bulbous enlargement. Stimulation of the sutured vagus nerve distal to the point of union had no influence upon the heart-rate. We were about to close the wound when the animal died as the result of careless anæsthetization.

Histological examination. — The vagus nerve just below the point of union is a solid mass of large medullated nerve fibres. The recurrent laryngeal nerve near its point of origin from the vagus consists of medullated fibres which appear to be somewhat smaller than those seen in the vagus. The fifth cervical nerve just central of the suture appears to be normal.

Dog No. 3. *Union of the peripheral end of the right vagus nerve with a branch of the right cervical plexus.* — Operation March 1, 1904. The second operation was performed 109 days after the nerves had been united (on June 17, 1904). The result of stimulation of the sutured nerve was totally negative so far as the rate of the heart-beat was concerned. But while the dog was lightly anæsthetized, sensory effects, such as hurried respirations and whining, were observed. Stimulation of the opposite vagus with the secondary coil at 14.5 gave complete cardiac inhibition.

During the course of this operation it occurred to us that the existence of the normal connection of the heart with the central nervous system through the intact vagus nerve of the opposite side might

[1] Erlanger: Johns Hopkins hospital reports, 1904, xii, p. 53.

exert some deleterious influence upon the establishment of new peripheral connections. Therefore a piece of the left vagus nerve 4 cm. long was excised. This was associated with the usual effects of section of both vagi, — namely, a marked increase in the heart-rate and a marked slowing of the respirations. The wound was then closed. After recovery from the anæsthetic, and until the animal's death five days later, the respirations remained slow and labored, and apparently none of the food ingested was retained.

At autopsy it was found that the sutured nerves had united nicely. The central stump of the vagus terminated in a bulbous enlargement.

Histological examination. — The vagus nerve some distance from the point of suture and the recurrent laryngeal nerve near its origin from the vagus contain medium sized medullated nerve fibres and very many nuclei.

Dog No. 6. *Union of the peripheral end of the right vagus nerve with a branch of the right cervical plexus.* — Operation March 4, 1904. The dog was found dead on October 14, 1904, *i. e.*, 224 days after the first operation. At autopsy it was found that the sutured nerves had united nicely. The central stump of the cut vagus nerve ended in a bulbous enlargement.

Histological examination. — The fifth cervical nerve, the vagus nerve distal to the point of union, and the recurrent laryngeal nerve contain large medullated fibres.

Dog No. 5. *Union of the peripheral end of the right vagus nerve with a branch of the right cervical plexus.* — This dog was a fully grown, active fox terrier. At the first operation, which was performed on March 3, 1904, the peripheral end of the right vagus nerve was united with what appeared to be the uppermost trunk of the right cervical plexus. The second operation was performed on December 31, 1904, 303 days after the first operation. At this time it was found that apparently good union had occurred between the sutured nerves. The central stump of the right vagus nerve had not grown down along the carotid artery. Stimulation of the right vagus nerve distal to the point of union with the cervical nerve resulted in partial inhibition of the heart with the secondary coil at 10. With the secondary coil at 6, complete inhibition was obtained, which, however, did not last longer than the time occupied by two heart-beats. The effect of stronger stimulation was not tested. Stimulation of the left vagus nerve with the coil at 12 gave complete

inhibition, from which the heart soon escaped. Upon cutting the left vagus nerve, the respirations at once became slow. The heart-rate was not materially affected. Thus before section of the left vagus nerve 54 pulsations were inscribed upon a given space of record; 55 pulsations were inscribed in the same space after section of the nerve. It was doubtful if reflex inhibition now occurred upon stimulation of the central end of the left vagus nerve. After excising a piece of the left vagus nerve the wound was closed.

Immediately after the operation the dog did well. The respirations were slow, but the animal seemed to be perfectly comfortable. Food was taken with apparent relish, and on the first day, at least, it was retained when given in small quantities. But, on the second day after the operation, retching and vomiting began and gradually increased in intensity. The weight of the animal increased for eleven days and then began to fall off. On January 20, twenty-one days after section of the left vagus nerve, and 324 days after the first operation, it was decided to make the final test, because it was thought the animal's appearance did not warrant further delay.

The animal was anæsthetized in the usual way, and the pressure in the femoral artery and the respirations were recorded. The sutured vagus nerve, including the neuroma at the point of union, was dissected free from the surrounding tissue and placed on shielded electrodes.

The results which follow demonstrate that, so far as its action on the heart is concerned, the sutured vagus nerve behaved in every respect like a normal vagus nerve.

In the first place it should be stated that stimulation of the central stump of the right vagus nerve did not slow the heart-rate.

By direct stimulation of the sutured vagus nerve any degree of inhibition could be obtained. In one trial the heart was held in complete inhibition for seventeen and three-fourths seconds before it escaped, and for more than a minute thereafter the heart-rate remained slow. The fall of blood-pressure that often follows the recovery from inhibition occurred regularly. Thus in one instance (Fig. 1) the mean blood-pressure before stimulation was 106.5 mm. Hg. Immediately after the heart had recovered from inhibition, the blood-pressure rose to 107, but fell secondarily to 75.

Weak stimulation of the central end of the left (unsutured) vagus nerve gave no perceptible changes in the heart-rate or blood-pressure. But strong stimulation of this nerve gave reflex slowing of the heart.

As is illustrated in Fig. 2, after a latent period of about two heart-beats, the heart-rate was distinctly slowed. Thus, before stimulation,

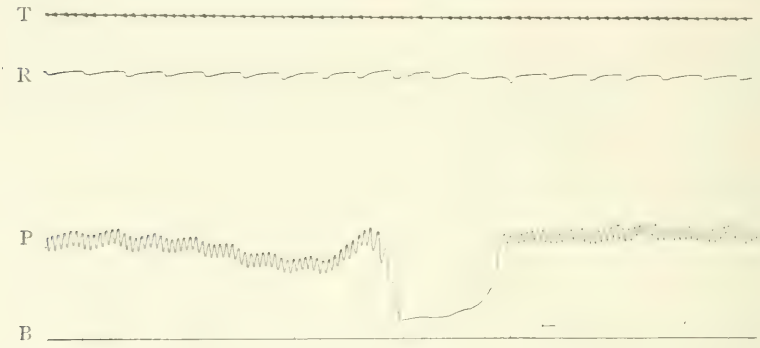


FIGURE 1. — One-fourth the original size. Showing the effect on the heart-rate, blood-pressure, and respiratory rate of stimulating the sutured vagus nerve. Dog No. 5. T = Time in seconds; R = Respiration; P = Pressure in femoral artery; B = Base line and signal.

23.5 pulse-waves were recorded in ten seconds (141 per minute). During the first 8.75 seconds of stimulation, 13 waves were recorded (89 per minute), but the rate gradually increased so that during the last five seconds of stimulation 11.5 beats were recorded (138 per minute).

The blood-pressure before stimulation was 87.5 mm. Hg. While the nerve was being stimulated, it rose to 122.5 mm. Hg; twenty

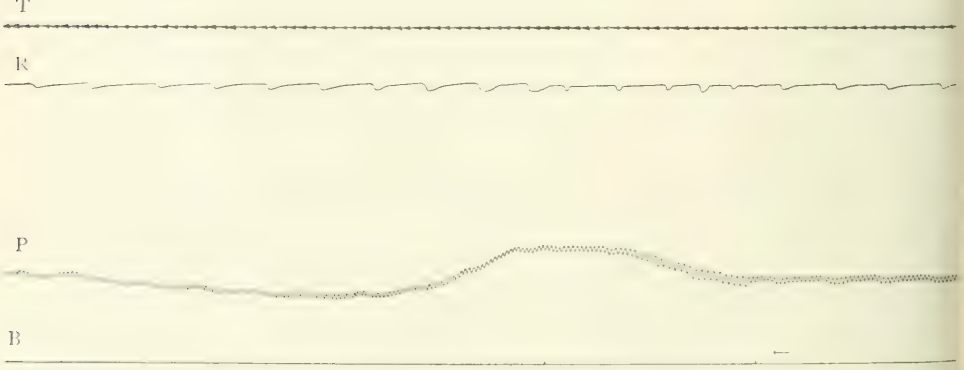


FIGURE 2. — One-third the original size. Showing the effect on the heart-rate, blood-pressure, and respiratory rate of stimulating the central end of the left vagus nerve. Dog No. 5. T = Time in seconds; R = Respiration; P = Pressure in femoral artery; B = Base-line and signal.

seconds after stimulation it was 71 mm. Hg; and it then gradually rose to the level it had had previous to stimulation of the nerve.

An effect of the respirations upon the heart-rate was perceptible in but limited regions of a long record. At such places the heart-rate during inspiration was distinctly faster than during expiration. An instance of this is shown in Fig. 3.

The central nervous system exercised a tonic inhibitory control over the heart through the sutured nerve. For when the sutured

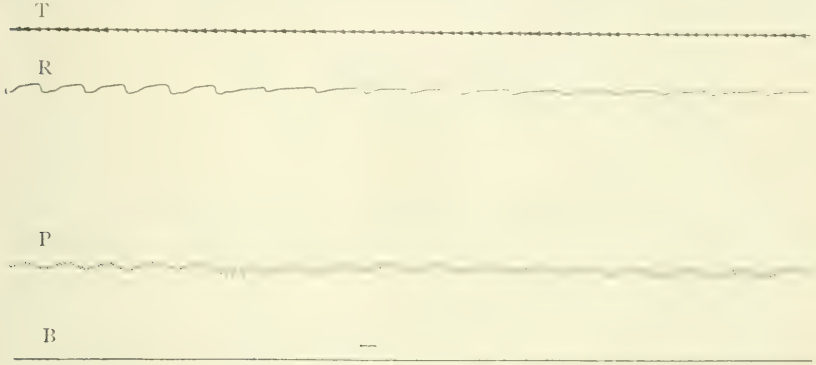


FIGURE 3. — One-fourth the original size. Showing the effect of the respiration on the heart-rate before and after ligation of the sutured nerve, and the effect of the ligation on the heart-rate. Dog No. 5. *T* = Time in seconds; *R* = Respiration; *P* = Pressure in femoral artery; *B* = Base-line and signal.

nerve was ligated (cut) the heart-rate, after a preliminary slowing, was distinctly increased (see Fig. 3). Immediately before ligation of the nerve the heart-rate was 66.5 to the half minute (133 per minute). Some time before ligation the rate was 68.5 per half minute (137 per minute). After the slowing which was associated with the mechanical stimulation of ligation had passed off, the heart-rate was 81 to the half minute (162 per minute). Before ligation of the nerve the blood-pressure was 96.5 mm. Hg. After ligation it gradually rose, and one and one-half minutes later it was 106 mm. Hg.

After ligation of the sutured vagus nerve, the effect of the respirations on the heart-rate described above disappeared (see Fig. 3). Furthermore, reflex inhibition of the heart could not now be obtained by stimulation of the central end of the left vagus nerve although thereby the blood-pressure was increased. Stimulation of the peripheral end of the sutured vagus still gave the usual cardiac inhibition. Stimulation of the central stump had no effect on the heart-rate, but it increased the blood-pressure.

The results obtained with regard to the relation of the sutured vagus nerve to the respiratory system were not definite. At the beginning of the experiment the respiratory rate was remarkably slow, 13.5 per minute. Just before section of the sutured vagus nerve, the animal was breathing 12 times per minute. Immediately after section of the sutured vagus nerve, the respiratory rate was 14 per minute, and this rate was maintained until the close of the experiment. Strong stimulation of the central end of the sutured vagus nerve increased both the rate and amplitude of the respirations. Stimulation with weak currents was without effect.

It was found at autopsy that the central stumps of both vagi were terminated by bulbous enlargements. They had made no attempt to grow down the neck. The branch of the cervical plexus which had been united with the peripheral end of the vagus nerve derived its fibres from the fifth and sixth cervical nerves, each of which sent a twig into it. The point of union of the sutured nerves was marked by a small fusiform swelling.[1]

Histological examination. — The following were removed for histological examination: A piece of the sutured vagus nerve about 1 cm. above the inferior cervical ganglion, a piece of the recurrent laryngeal nerve, and a piece of the fifth cervical nerve. The vagus and recurrent laryngeal nerves were found to consist of solid masses of uniformly large medullated fibres. The fifth cervical nerve appeared to be perfectly normal.

Dog. No 4. *Union of the peripheral end of the right vagus nerve with a branch of the right cervical plexus.* — March 11, 1904. Dog No. 4 was a large but young mongrel. On January 24, 1905, *i. e.*, 319 days after the first operation, a preliminary operation was performed in order to determine whether or not functional regeneration had occurred. The portion of the vagus nerve that had been united with the cervical nerve was placed on shielded electrodes.

[1] With regard to the viscera the following note may be made. The lungs and heart were normal. The œsophagus was not especially dilated. It contained material which was similar to the stomach contents. The cardiac orifice was patulous, so that there was perfectly free communication between the œsophagus and the stomach. The stomach was relaxed and distended with a bile-stained material of a semi-fluid consistency in which a granular material and rather large pieces of bone were suspended. The pylorus was so tightly contracted that considerable pressure was required to express the gastric contents into the duodenum. The small intestines were relaxed and almost empty. All but the uppermost 3 cm. of the large intestines were filled with a hard scybalous mass.

As indicated by the sphygmomanometer attached to the animal's leg, partial inhibition of the heart was obtained when the nerve was stimulated with the secondary coil at 8 and at 6. A piece of the left vagus nerve 3 cm. long was then removed. Now stimulation of the sutured nerve gave but very slight inhibition. The results obtained at this preliminary test were not so decided as were those obtained at the similar test made on Dog No. 5.

The wound healed quickly, and the sutures were removed on the seventh day. But the animal did not do well. Food given by mouth was retained but a very few moments. Retching and vomiting were almost incessant. When neither food nor drink was given, the dog vomited a stiff, frothy material which probably consisted of swallowed saliva.[1]

In nine days following the operation the animal lost over 3 kgm. in weight. At this time his condition appeared to be so serious that it was decided to make the final test for regeneration, although it was surmised that a sufficient interval of time had not elapsed since the section of the left vagus nerve to permit of a readjustment to the new conditions.

The methods used in the final tests follow. A continuous record was made of the pressure in the femoral artery, of the respirations and of the time in two second intervals. The positions of the stimulations were indicated with a stimulating marking pen, which was also used to inscribe the base-line. After obtaining a normal record, the effect was tested of stimulating with the induced current the sutured (right) vagus nerve distal of the point of union, the central stump of the left vagus nerve, and the sixth cervical nerve of the left side. The central stump of the cervical nerve that had been united with the vagus was too short to be dissected out and stimulated. Then an attempt was made to establish a block to the passage of impulses through the sutured nerve by freezing it. For this purpose the following simple device was used. A piece of glass tubing of 5 mm. bore was bent sharply on itself until the two arms were parallel to one another and

[1] An attempt to feed the animal through a stomach tube resulted in failure. Apparently the tube passed into the stomach without meeting with any obstruction, but the material injected was immediately regurgitated. Then rectal feeding was begun, but although the enemata were well retained, the animal continued to lose in weight. As the vomitus was always of an alkaline reaction, it was thought that the addition of an acid to the food given by mouth might help to empty the stomach through the pylorus. But this likewise was of no avail.

TABLE OF RESULTS.— Dog No. 4.

Remarks.	Procedure.	Pulse-rate per min.	Blood-pressure.		Respirations per min.
			Max.	Min.	
Normal at beginning of exp.		147.6	123	94	11.5
(Period of stim. too brief for estimation of resp. rate)	Before	133.7	117	89	
	Stim. sutured vagus: coil at 11				
	During	115.8	116	82	Rate incr. slightly.
	Before	143.6	128	97	
	Same: coil at 9				
	During	117.4	100	80	
	Before	150.0	124	96	
	Same: coil at 8				
	During	112.9	113	80	Rate incr. markedly.
	Before	143.2	126	98	
	Same: coil at 6				
	During	126.8	106	83	Same.
					10.5
	Before	129.7	121		
	Stim. central end left vagus: coil at 10				
	During	126.0	169		Exp. tetanus.
	Before	140.5	148		
	Same: coil at 8				
(Period of slowest pulse-rate)	During	127.6	211		Exp. tetanus then incr. rate.
	Before	128.6	152	138	
	Stim. left 6th cerv. coil at 12				
	During	108.9	136	118	Rate incr. slightly.
	Before	121.6	134	117	
	Same: coil at 10				
	During	109.6	126	103	Same.
	Before	124.9	127	106	
	Same: coil at 8				
	During	112.9	118	96	Same.
					6.5
Test before cooling sutured nerve	Before	141.6	108	91	
	Stim. sutured vagus: coil at 7				
	During	120.0	98	80	Rate incr. markedly.

TABLE OF RESULTS. — Dog No. 4 (*Continued*).

Remarks.	Procedure.	Pulse-rate per min.	Blood-pressure.		Respirations per min.
			Max.	Min.	
Normal just before cooling		126.4	114	92	7
Nerve cooled to 1° C.		134.0	121	103	10
	Before	134.2	121	103	
Nerve cooled	Stim. sutured vagus: coil at 7				
	During	130.3	101	86	No change.
	Before	129.2	127		
Nerve cooled	Stim. left vagus: coil at 7				
	During	133.3	196		Exp. tetanus then rate incr.
	Before	129.3	132	117	
Nerve cooled	Stim. left 6th cerv.: coil at 7				
	During	126.6	114	100	Rate incr. slightly.
Nerve warmed: normal		124.0	116	100	7.5
Nerve warmed	Stim. sutured vagus: coil at 7	104.4	85	73	Exp. tetanus.
Some bleeding: resp. stopped: art. resp. during rest of exp.					
	Before	109.5	106	90	
Before cutting cord	Stim. sutured vagus: coil at 7				
	During	101.7	86	72	
	Before	116.2	112	102	
	Same: coil at 7				
	During	103.6	95	82	
				Mean	
	Before	104.5		42	
Cord cut in upper cervical region	Same: coil at 7				
	During	80.0		44	
	Before	97.9		40	
	Same: coil at 7				
	During	88.2		42	
	Before	94.4		Not appreciably changed.	
	Stim. left 6th cerv.: coil at 7				
	During	93.2			
	Before	90.0			
	Same: coil at 5				
	During	94.0	Same.		

TABLE OF RESULTS.—Dog No. 4 (*Continued*).

Remarks.	Procedure.	Pulse-rate per min.	Blood-pressure.		Respirations per min.
			Max.	Mean.	
	Before	104.3		32	
	Stim. central end left vagus: coil at 5				
	During	105.4		34	
	Before	97.8		30	
	Stim. left 7th cerv.: coil at 5				
	During	97.0		32	
Before cooling sutured nerve		96.0		30	
After cooling		73.0		26	
Nerve cooled	During stim. sutured vagus: coil at 7	66.3		30	
	Before	71.2		26	
Nerve cooled	Stim. sutured vagus: coil at 7				
	During	67.5		30	
After warming		79.2		24	
	Stim. sutured vag.	62.1		28	
	Before	80.3		24	
	Same: coil at 7				
	During	63.6		27	
Nerve cooled		66.4		24	
Nerve cooled	Same: during stim.	64.6		26	
	Before	66.5		22	
	Same: coil at 7				
	During	66.0		26	
After warming		77.4		25	
	Same: during stim.	59.3		27	
	Before	78.5		25	
	Stim. left 7th cerv.: coil at 7				
	During	79.3		27	

separated by an interval of 2 mm. One arm of this U tube was then cut off within 6 mm. of the concavity of the bend. After the nerve had been slipped into the concavity of the bend, the inflow tube was

fastened into the short arm of the U tube. The temperature of the circulating medium was determined as it issued from the cooling tube.

This cooling apparatus was placed on the sutured nerve just distal of the shielded electrodes with which the nerve was stimulated. After having cooled the nerve to 1°–0.5° C., the order of stimulation that had been employed before cooling was repeated. The results obtained are given on pages 382 to 384 in tabular form.

Upon inspection of this Table, it may be seen that undoubted partial inhibition of the heart was obtained both by direct stimulation of the

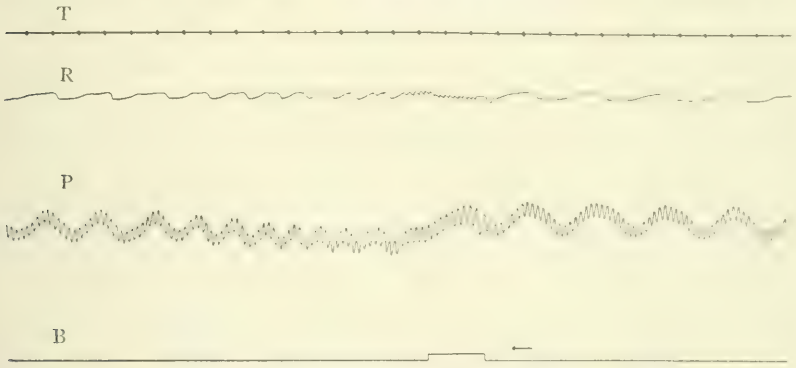


FIGURE 4. — One-third the original size. Showing the effect on the heart-rate, blood-pressure, and respiratory rate of stimulating the sutured vagus nerve. Dog No. 4. *T* = Time in 2 seconds; otherwise legend same as in preceding figures.

vagus nerve that had been sutured to the cervical nerve (Fig. 4) and reflexly, through this nerve, upon stimulation of the central stump of the opposite vagus nerve (Fig. 5), and of the cervical nerve opposite to the one that had been sutured to the vagus nerve.

It was not possible to obtain complete inhibition of the heart by stimulating the sutured nerve, but thereby the heart-rate was materially reduced. Special attention should be called to the fact that before section of the cord stimulation of the sixth left cervical nerve gave reflex inhibition which was fully as marked as, if not more marked than, the reflex inhibition that resulted from stimulation of the central stump of the left vagus nerve. Thus with the secondary coil at 12 (not included in the Table) and at 10, stimulation of the central stump of the left vagus nerve produced practically no inhibition of the heart. The maximum effect, which was obtained with the coil at 8, was a reduction of the heart-rate from 140.5 to 127.6 beats per

minute. Stimulation of the sixth left cervical nerve with the coil at 12 reduced the heart-rate from 128.6 to 108.9 beats per minute. When the strength of the stimulus was increased, the inhibitory effect diminished, but it still remained as marked as that obtained by stimulation of the central end of the vagus. Thus, with the coil at 10, the rate was reduced from 121.6 to 109.6, and with the coil at 8 it was reduced from 124.9 to 112.9 beats per minute.

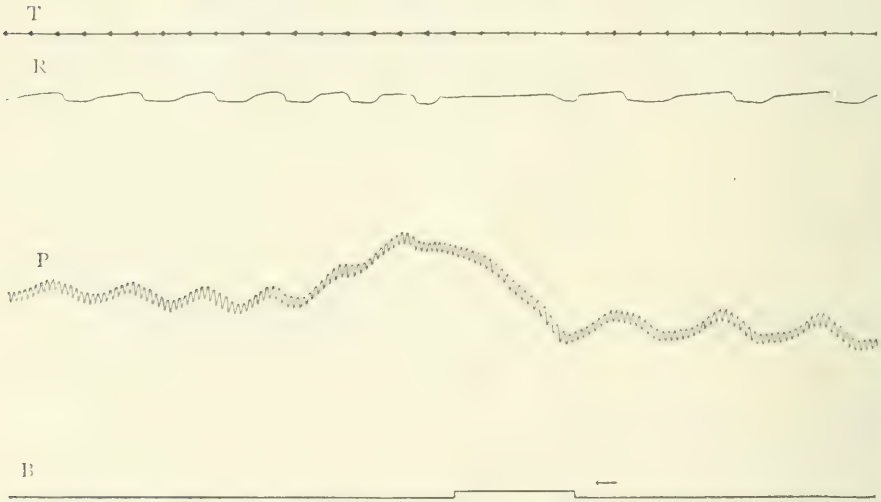


FIGURE 5. — One-fourth the original size. Showing the effect on the heart-rate, blood-pressure, and respiratory rate of stimulating the central end of the left vagus nerve Dog No. 4. Legend same as in Fig. 4.

When the sutured nerve was cooled to 1° C., there was good reason for believing that functional connection of the heart with the central nervous system through this nerve was practically severed. For now stimulation of the sutured nerve reduced the heart-rate less than 4 beats per minute, whereas, before cooling, stimulation had regularly reduced the heart-rate more than 20 beats per minute. The effect of such functional section of the sutured vagus was to increase the heart-rate from 126.4 beats per minute to 134. Subsequently, upon warming the nerve, the rate became 124. It would, therefore appear, although the result is by no means striking, that the central nervous system had assumed tonic inhibitory control over the heart through the sutured nerve.

The effect upon the blood-pressure of cooling the sutured vagus nerve may perhaps be taken as additional evidence of the existence of such tonic activity. For before cooling and after warming the

nerve the pressures were 114–92 and 116–100, respectively, and while the nerve was cooled, it was 121–103 mm. Hg.

Cooling the sutured nerve practically stopped all reflex cardio-inhibition which before cooling had been obtained upon stimulation of the central stump of the left vagus nerve and of the sixth left cervical nerve. The slight effects upon the heart-rate here seen probably fall within the error of the methods employed. The effects upon the blood-pressure can be of no assistance in this case, because they may have resulted through action of the stimuli upon the vasomotor centres.

The results obtained after cutting the cord were totally negative in so far as they concern the influence exerted by the cord upon cardio-inhibition. Section of the cord had no perceptible effect upon the heart-rate. It is true that the number of beats per minute was smaller after section than before. But examination of the table will make it appear probable that the reduction of the heart-rate seen here is merely a progressive continuation of the slowing that had begun earlier in the experiment. However, the fact that section of the cord was not followed by cardio-acceleration might at first sight seem to indicate that the cord had exercised no inhibitory control over the heart, or, in other words, that the medulla was to be held responsible for the tonic inhibition which the earlier experiments had demonstrated to be present. But this view is not supported by the facts. For after section of the cord, functional section of the sutured vagus nerve by cooling did not increase the heart-rate; indeed, it had just the opposite effect. Thus, upon alternate warming and cooling of the sutured nerve, the following heart-rates were recorded: 96, 73, 79.2, 66.4, and 77.4. This result is sharp and incontestable. However, we have not the data upon which a satisfactory explanation might be based. That the slowing of the heart-rate was the result of the slight rise of blood-pressure which was always associated with the stimulations, seems improbable.

It is also worthy of remark that, after section of the cord, stimulation of the sutured nerve, whether cooled or not, causes a slight but constant rise of the blood-pressure, this rise being approximately the same, irrespective of the effect upon the heart-rate.

After the cord had been cut, it was still possible to obtain well-marked cardio-inhibition by direct stimulation of the sutured vagus nerve. But now stimulation of the left sixth and seventh cervical nerves and of the central stump of the left vagus, gave no reflex slowing of the heart.

It is interesting to note that in this experiment, as well as in the other successful experiment of this investigation, the normal influence of the respiration on the heart-rate is occasionally seen. Thus at one place, in equal intervals of time, 8.5 heart-beats were regularly recorded during expiration to 9 beats during inspiration.

Likewise, in this animal, the presence in the sutured nerve of sensory fibres influencing the respiratory centre was demonstrated. Thus by stimulation of the sutured nerve it was possible to elicit both expiratory tetanus and an increase in the respiratory rate (see the figures). But, as in the case of Dog No. 5, these sensory fibres had not acquired their normal function of regulating the respiratory rate. For when, at the second operation, the left vagus nerve was cut, the respirations at once became slow and deep. This condition persisted at the time of the final operation, nine days later. At the beginning of the experiment, the respiratory rate was 11.5 per minute. At a later period, it was 6.5 per minute. Immediately before cooling the sutured nerve, it was 7 per minute. Upon cooling the nerve, it increased to 10 per minute, returning to 7.5 when the block was removed. Therefore, in both experiments, functional severance of the sutured nerve increased the rate of respirations. As this phenomenon was not associated with any constant change in the blood-pressure, it is impossible to account for it from known facts.

In the case of neither animal was there any obvious evidence of a restoration of the normal innervation of other organs in which the vagus nerve is distributed. The bark of all the animals remained hoarse, and in those in which the left vagus nerve was severed persistent retching and vomiting indicated either that the regenerating fibres had not yet reached the organs of the gastro-intestinal canal, or that, if they had grown into them, they had not assumed the normal functions of vagus fibres. It is also interesting to note that although, after section of the second vagus nerve, the appetite remained unimpaired, the vomitus which, from the evidence we have obtained, apparently came from the stomach, always had an alkaline reaction.

At autopsy it was found that the nerve that had been sutured to the peripheral end of the vagus was one of the two trunks of the sixth cervical nerve. The point of union was marked by a spindleformed swelling. This was considerably larger than the one found in the case of Dog No. 5, indicating that the suture had not been as neatly made. It is possible that this may have accounted for the greater intensity, in Dog No. 4, of the symptoms which followed section of

the left vagus, and for the impossibility of obtaining complete cardio-inhibition by stimulation of the sutured nerve.

A neuroma about the size of a pea had developed on the central stump of the right vagus nerve at the place of section. From the distal end of this neuroma a tapering projection, about 3 cm. long, extended peripherally along the course of the carotid artery. The base of the projection was about 1 mm. in diameter, and had the appearance of a nerve trunk. Peripherally it seemed to fork, although at this point it became so frail that it was difficult to distinguish it from the surrounding connective tissue. One branch appeared to follow the carotid artery for a short distance, but every trace of it had disappeared while still at a distance of about 4 cm. from the neuroma on the sutured nerve. The other branch seemed to pass into the sterno-mastoid muscle. Two pieces of this outgrowth, one just distal of the neuroma, the other 1 cm. further from it, together with a piece of the sutured nerve near the inferior cervical ganglion, were removed for histological examination.[1]

Histological examination. — The piece of the sutured vagus nerve taken from a point near the inferior cervical ganglion was made up largely of connective tissue through which there was scattered a considerable number of large medullated nerve fibres. The proximal piece of the outgrowth from the central stump of the right vagus nerve was composed of medullated fibres which were somewhat smaller than those seen in the sutured nerve. The tissue taken from the more distal position contained no nerve fibres.

Dog No. 1. *Union of the central end of the right hypoglossal nerve with the peripheral end of the right vagus nerve.* — At the first operation, which was performed on February 24, 1904, the hypoglossal and vagus nerves were sewn together, and a piece of the central stump of the vagus about 1.5 cm. long was excised. After the operation the dog developed a severe keratitis, but otherwise his recovery was uneventful.

[1] *Post-mortem* examination of the rest of the organs was negative. The œsophagus was somewhat dilated. The fundic portion of the stomach was contracted and contained a small amount of a bile-stained fluid of an alkaline reaction. The cavity of the stomach was in free communication with the lumen of the œsophagus which was filled with the same material seen in the stomach. The pylorus was so tightly contracted that the gastric contents could be expressed into the duodenum only with the greatest of difficulty. The gall-bladder was distended with bile. The intestines presented nothing remarkable. The lungs and heart were apparently normal.

On May 14, 1904, eighty days after the first operation, the sutured nerve was exposed below the point of union, and stimulated. The result was totally negative. On February 9, 1905, 351 days after the first operation, the physiological test for regeneration was repeated, but the results were again negative. No slowing of the heart-rate was obtained with the secondary coil at 0. And with the secondary coil at 5 the rate of the respirations was not affected. Stimulation of the left (unsutured) nerve with the secondary coil at 15 gave complete inhibition of the heart.

A third test was made on March 15, 1905, 385 days after the nerves had been united, and again the results were totally negative. At this operation a piece was excised from the vagus nerve of the opposite side. Section of this nerve was associated with a slowing of the respirations and an increase of the heart-rate. After this operation the dog did quite well; indeed, very much better than any of the other dogs in which both vagi had been cut. Although this dog had more or less constant vomiting, the vomitus was always reingested, and finally it was retained. In twenty-one days the animal lost but 0.8 kgm. in weight. At this time the respiratory rate was 10 per minute.

On May 5, 1905, 406 days after the first operation, stimulation of the sutured nerve again gave totally negative results. The dog died on the table after all of the tests had been made.

At autopsy it was found that the central stump of the vagus nerve had made no visible attempt to grow down the neck. The usual neuromata were found, one at the place of union of the sutured nerves, the other on the central stump of the vagus nerve. These were separated by a distance of about 1.5 cm., and were connected by some dense tissue. This tissue, together with two pieces of the sutured nerve, one taken from a place near the point of union, the other from a place near the inferior cervical ganglion, were removed for histological examination.[1]

Histological examination. — The tissue obtained from this dog was fixed in osmic acid and teased on the slide. In the tissue that united the two neuromata was found a bundle of medullated fibres. The size of the fibres was very variable; roughly, the frequency of

[1] The œsophagus was somewhat dilated, but the cardiac orifice of the stomach, as well as the pylorus, were closed. The stomach was distended with large pieces of undigested meat. In the left ventricle was found a comparatively fresh, large white thrombus.

large and small fibres was approximately equal. As the bundle was torn in teasing, no estimate could be made of its size, but it undoubtedly contained a considerable number of fibres.

The hypoglossal vagus nerve near the point of union contained medullated fibres, most of which were of the large variety. Likewise, medullated fibres were found in it near the inferior cervical ganglion, but here most of the fibres were of the small variety.

Although the results of this experiment are negative, in so far as they concern functional regeneration, still they are of interest in at least two particulars. In the first place, they serve to indicate that, in experiments on cross-suturing of nerves, resection of a piece of a central stump which is left free in the wound may not suffice to prevent a re-establishment of normal innervation.

In the second place, it is interesting to note that, although vagus fibres had undoubtedly united with the sutured nerve, they had not yet assumed control of vagus functions. This apparent sluggishness might be accounted for in one of three ways: (a) It is of course possible that the connection of vagus with vagus had not been established long enough to allow of functional regeneration. (b) Vagus fibres might not regenerate as rapidly as other fibres. (c) The presence in the regenerating nerve of fibres derived from two sources might in some way have interfered with the process of regeneration. The data of this experiment do not suffice for a discussion of the relative importance of these possibilities.

Summary and Discussion.

The results of the experiments herein described demonstrate that when the central end of a spinal nerve is sewn to the peripheral end of the vagus nerve the fibres of the former may make functional connection with the vagus end-apparatus in the heart, if sufficient time be allowed for regeneration. Complete inhibition of the heart may be obtained by direct stimulation of such regenerated fibres. Such fibres may serve as the efferent path of cardiac reflexes which are associated with the act of respiration, and of reflexes started by electrical stimulation of afferent nerves. And finally through these fibres the central nervous system may exercise a tonic inhibitory control over the heart.

In one of the instances of successful union, an attempt was made to locate the inhibitory centre, that is, to determine whether the reflex centre still maintained its normal position in the medulla, or

whether it had moved to the cells of origin of the spinal nerve which was performing the functions of the vagus. It will be recalled that, after cutting the cord in the upper cervical region, stimulation of the central end of the unsutured vagus nerve or of the sixth cervical nerve, no longer caused reflex cardiac inhibition. But this does not prove that the reflex centre had not moved into the cord. Nor is the situation of the centre in the medulla proved by the fact that after transsection of the cord in the upper cervical region no acceleration of the heart-rate followed functional section of the sutured nerve. For it is possible that transsection of the cord may have produced spinal shock, a condition in which, according to Sherrington,[1] the portion of the cord aboral of the mechanical injury suffers a more or less complete suppression of nervous function.

The only evidence which favors the location of the reflex centre in the cord consists in the observation that reflex inhibition of the heart was more easily obtained by stimulation of a cervical nerve than by stimulation of the central end of the vagus nerve. However, this evidence can be considered suggestive only, for under normal circumstances reflex inhibition of the heart may be obtained by stimulation of any afferent nerve in the body.

As these experiments have failed to locate positively the so-called cardio-inhibitory centre, a discussion of the way in which the central nervous system has adapted itself to the changed conditions resulting from union of a spinal nerve with the vagus nerve could not be expected to lead to a positive conclusion. Nevertheless a discussion of the possibilities might not be out of place.

From what is known of regenerative processes within the central nervous system, it is highly improbable that axons of the cells of the vagus centre could grow through the cord and cervical nerve and so reach the heart. And there is no evidence, even if we admit Bethe's hypothesis,[2] that a growth of fibres could occur along the same course in the reverse direction.

But it is possible that the resistance to the passage of impulses along descending fibres, which probably connect the vagus centre with spinal nuclei, might have been diminished as a result of their unwonted activity. In this way they might have become the natural path of discharge of the normal cardio-inhibitory centre.

[1] Sherrington: Schäfer's Text-book of physiology, London, 1900, ii, p. 846.

[2] Bethe: Allgemeine Anatomie und Physiologie des Nervensystems, Leipzig, 1903, p. 182.

Finally it might be assumed, and it will be remembered that we have obtained some evidence in favor of this view, that the inhibitory centre has actually taken its abode in the nucleus of origin of the cervical nerve. In this connection attention should be called to the opinion first expressed by Bernstein [1] that the tone of the inhibitory centre is probably not the result of automatic activity of the centre, but rather the result of the continual arrival in it of afferent impulses. Whether or not the cardio-inhibitory centre selects and adapts afferent impulses so as to make them subserve the best interests of the organism, it is impossible to state. The great differences in the response of the inhibitory centre to stimulation of various afferent nerves might, perhaps, be taken as evidence of such a selective action. But here it is again possible that, through inheritance or exercise, certain tracts have become the paths of least resistance. It is, therefore, not necessary to assume that the cardio-inhibitory centre possesses automaticity. The facts do not debar the view that the continual variations in the tone of the cardio-inhibitory centre are the result merely of the arrival and spread of impulses of all kinds in the central nervous system. And it might, in addition, be admitted that when the vagus nerve connects the heart with the central nervous system, this spread of impulses, modified to some extent by the resistance offered to their passage along the various paths, affects the heart through the cells of origin of the vagus fibres. But should the axis cylinders of any other group of cells in the central nervous system make connection with the inhibitory mechanism in the heart, then the spread of impulses might affect the heart in the same way and with an intensity which might vary with the efficiency of the impulses acting upon the cells of origin of the nerve fibres. In other words, such a group of cells might become the reflex centre for cardio-inhibition. Therefore, in the case of the cardio-inhibitory centre it would not be necessary to conclude with Rawa [2] that "the nerve centres will perform just those functions which are demanded of them by the peripheral organs with which they are connected."

Our observation that the transmission of reflexes to peripheral organs along fibres which do not normally make connection with such organs is not unique. As has been stated, Rawa's instances are not perfectly clear. It is possible that in his experiments vagus

[1] Bernstein: Archiv für Anatomie und Physiologie, 1864, p. 633.

[2] Rawa: *Loc. cit.*

fibres may have resumed their normal connections. But Mislavsky[1] has undoubtedly obtained reflex contraction of the laryngeal muscles after union of the thoracic end of the cervical sympathetic with the recurrent laryngeal nerve. Perhaps the most striking instance of this kind is seen in the case reported by Cushing,[2] in which, after union of the central end of the spinal accessory nerve with the peripheral end of the facial nerve in man, the subject gradually regained voluntary control of individual facial muscles. It should be stated here that Cunningham[3] maintains that in the dog normal control is never regained over abnormally innervated voluntary muscles. Furthermore, the recovery of voluntary control in such a case might not be comparable to the recovery of a reflex control. In the former case the recovery of function might be facilitated by training; in the latter case training could not play a part.

Our experiments do not permit of the formulation of a positive conclusion with regard to the rate of regeneration of fibres of different origin when united with the vagus nerve. Nevertheless, when viewed in conjunction with the experiments of others, they do indicate that spinal fibres make functional connection more rapidly than fibres of the hypoglossal and vagus nerves. In our most successful case, complete inhibition of the heart was obtained 303 days after the cervical and vagus nerves had been sutured. In one instance of union of the hypoglossal (and vagus?) with the vagus nerve, no signs of functional regeneration were obtained 406 days after sewing the nerves together. However, Henri and Calugareanu[4] describe one instance in which complete inhibition of the heart was obtained 170 days after the same operation. Many investigators have tested the power of the vagus to regenerate itself. All final tests made within a year of the operation have yielded negative results.[5]

Neither has conclusive evidence been obtained with regard to the influence which an intact vagus nerve exerts upon the rate with which a regenerating vagus nerve will make functional connection with the end apparatus in the heart. However, one fact seems to indicate that it has a retarding influence. At the last preliminary operation

[1] Mislavsky: Comptes rendus de la société de biologie, 1902, liv, p. 841.

[2] Cushing: Journal of nervous and mental diseases, 1903, xxx, p. 367.

[3] Cunningham: This journal, 1898, i, p. 239.

[4] Henri and Calugareanu: *Loc. cit.* These authors do not give the results of *post-mortem* examinations.

[5] Tuckett: *Loc. cit.*

in Experiment 5, it was found that strong stimulation of the sutured nerve had but a slight effect upon the heart-rate. The inhibition that resulted did not last longer than the time occupied by two heart-beats. Only twenty-one days later strong stimulation of the sutured nerve held the heart in complete inhibition for seventeen and seven-tenths seconds. It is rather improbable that this sudden change in the response of the heart to vagus stimulation would have occurred in the course of an uninfluenced regeneration.

Finally, it might not be superfluous to state that the fact that impulses carried to the heart through spinal fibres may bring the heart to rest, favors the view that cardiac inhibition results from the action of ordinary nerve impulses upon a peculiar end-apparatus.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE HEART.—II. THE FUNCTION OF THE CARDIAC NERVES IN MOLLUSCS.

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Introduction.

THE work on the physiology of the heart nerves of invertebrates was undertaken with the view of determining the nature of the inhibition of the invertebrate heart on direct stimulation with the induced current. Single induced shocks, as well as the interrupted current of a certain intensity, when applied directly to the heart of molluscs, cause cessation of the rhythm in diastole, together with relaxation of the musculature greater than that during the normal diastolic pause. Is this inhibition produced by direct action of the electrical current on the muscle, or by the stimulation of inhibitory nerves and nerve-endings in the heart? The former is the view taken by Foster,[1] based on his work on the heart of the snail (*Helix*). The latter is the theory upheld by Ransom [2] based on his experiments on the heart of several molluscs, particularly on the systemic ventricle of *Octopus*. That the systemic heart of cephalopod molluscs is provided with inhibitory nerves has been known since the observations of Bert on *Sepia*, and of Fredericq on *Octopus*.[3] Ransom found that

[1] Foster: Archiv für die gesammte Physiologie, 1872, v, p. 191; Proceedings of the Royal Society, 1875, xxiii, p. 318.

[2] Ransom: Journal of physiology, 1884, v, p. 261.

[3] Fredericq: Archives de zoologie expérimentale et générale, 1878, vii, p. 535.

the heart of *Helix* is similarly provided with inhibitory nerves. The force of Ransom's argument that the inhibitory effects observed on direct stimulation of the heart are due to stimulation of inhibitory nerves is this, that he obtained inhibition on direct stimulation of hearts provided with inhibitory nerves, and that in the case of hearts not provided with inhibitory nerves he failed to obtain inhibition on direct stimulation. Before I became acquainted with Ransom's work, I had obtained inhibitory effects on direct stimulation of the heart of molluscs in which Ransom failed to find them, and particularly in the gill ventricles of the cephalopods. Ransom not only failed to obtain inhibition on direct stimulation of the branchial ventricles, but he claims that these ventricles are provided with accelerator nerves only. This made a re-examination of the question desirable. If the inhibition of the heart on direct stimulation with the induced current is due to stimulation of inhibitory nerves, my results showed that the heart of molluscs, crustaceans, and probably also the tunicates, must be provided with inhibitory nerves. These nerves ought to be discoverable both anatomically and physiologically.

From what has been said, it is plain that I set out to determine the nature of the inhibition of the heart on direct stimulation, accepting the myogenic theory of the heart-beat. I did not question the applicability of the myogenic theory for the heart of invertebrates, until in Limulus I was brought face to face with anatomical conditions which rendered crucial experiments touching the nature of the heart-beat possible. The nervous origin of the heart-beat placed the question of the nature of the inhibition of the heart on direct stimulation in a different light, as the electrical current may act on the ganglion cells in a way to stop their activity, or on the nerve-endings in the muscle, or on the muscle itself, in a way to block the nervous impulse or render the muscle unable to respond. The activity of the ganglion on the Limulus heart can be inhibited by direct stimulation with the interrupted current even after the influence of the inhibitory nerves have been abolished by the action of certain drugs. This fact, it appears to me, furnishes the key to the solution.

It has been the aim to include in this work as many representatives of the different groups of invertebrates as could be obtained. A grant from the Carnegie Institution enabled me to work on marine invertebrates both on the Pacific (Monterey Bay, San Diego Bay), and the Atlantic coast (Woods Holl).

For descriptions of the anatomy of the cardiac nerves of the inver-

tebrates included in this and subsequent papers, the reader is referred to Part I, Biological Bulletin, 1905, VIII, p. 123.

Experimental Methods.

For greater accuracy in studying the function of the cardiac nerves it is, of course, necessary to record by some direct graphic method the changes, if any, in rhythm and form of the cardiac muscle produced by stimulation of the nerves. The method of studying the changes in the cardiac muscle by recording the changes in the arterial blood pressure is not at all applicable to the invertebrates, for the reason that, with the exception of the larger octopi and the giant squids, the hearts are too feeble and the necessary dissections cause too much loss of blood, as the vascular system is partly lacunar. Fuchs[1] has, to be sure, made use of this method in studying the physiology of the cardiac nerves in *Octopus*, but in order to avoid too much loss of blood he did not sever the branches of the visceral nerves which supply other muscular organs besides the cardiac apparatus. Hence when he stimulates the visceral nerves close to the brain, and observes the attendant changes in blood pressure, it becomes simply impossible to tell how much of these changes are brought about by *active* changes of form of the vascular organs themselves, and how much is due to *passive* changes of form caused by contraction of other muscles.

In the decapod crustaceans, the movement of the heart can be fairly accurately recorded with the heart *in situ*, by aid of an upright placed on the dorsal pericardium or directly on the dorsal side of the heart; but in the lamellibranchs and most of the gasteropod molluscs, the walls of the ventricle are not sufficiently muscular to allow this procedure. I obtained the most satisfactory results by isolating the hearts, together with the heart nerves, and suspending the heart in such a way that the cardiac nerves were not interfered with. In the chitons, I did not succeed in isolating the ventricles, in order to suspend them without destroying the physiological continuity of the nerves. The auricles of most of the molluscs are almost too delicate for suspension, and that is also true of the gill ventricles of *Loligo*. For the hearts or parts of the hearts in which the influence of the nerves could not be studied in this way, direct observation had to suffice. But instead of measuring the variations in

[1] Fuchs: Archiv für die gesammte Physiologie, 1895, lx, p. 173.

the rate of the beats by aid of the second hand of a watch, I recorded the beats on the kymograph by means of an electro-magnetic signal. The key in the circuit was worked by the hand of the observer. Now if the writing point of the signal was arranged perpendicularly above or below that of the signal in the primary circuit of the induction coil, and the usual time marker added, indirect myograms were obtained, which recorded slight variations and peculiarities in the rhythm much more accurately than was possible by simple direct observation. This device was made use of by Foster and Dew-Smith (1875) in studying the response of the heart of *Helix* to electrical stimulation. I have made use of the method, not only in studying the physiology of the cardiac nerves, but also for recording the effect of direct stimulation of the auricles and ventricles that were too feeble for suspension, and I will refer to it as the "indirect graphic method."

The dissections required for isolating the cardiac nerves and exposing the heart so that its movements can be observed and recorded, involve almost invariably such extensive bleeding that the heart is emptied of blood. Artificial filling of the ventricles or the auricles can usually not be accomplished without injury to the nerves to the aortic and the auricular ends of the heart.

No anæsthetics were employed in preparing the animals for the experiments.

The Lamellibranchs.

A cursory study of the influence of the nervous system on the heart of clams has been made by Yung[1] and Buddington.[2] Yung worked on *Mya*, *Anadonta*, and *Solen*. On stimulation of the branchial or visceral ganglion with the interrupted current, he observed a momentary arrest of the heart, immediately followed by a great augmentation of the rhythm; from which he concludes that the nerves which pass from the ganglion to the heart have an accelerator function: "Ces nerfs jouent le rôle d'agents accélératéurs des mouvements cardiaques, de telle manière que leur excitation augmente le nombre des pulsations et leur rupture diminue ce nombre" (p. 428). Buddington worked on *Venus*, concluding that the heart of this lamellibranch is provided with inhibitory nerves from the visceral ganglion.

[1] Yung: Archives de zoologie expérimentale et générale, 1881, ix, p. 429.

[2] Buddington: Biological bulletin, 1904, vi, p. 311.

My own work was done on *Mytilus californianus*, *Mytilus edulis*, *Mya arenaria*, *Tapes staminea*, *Platydon cancellatus*, *Venus mercenaria*, *Cardium quadrigenerium*, *Hennites gigantea*, *Pecten irradians*. Some of these are quite far apart in the systematic grouping. Fortunately two of them (*Mya*, *Venus*) are the same as those worked on by Yung and Buddington. Leaving out of consideration for the present the condition in *Mytilus*, it may be stated on the outset that the experiments on all the other lamellibranchs show the presence of cardio-inhibitory nerves, and apparently of these nerves only. I have failed to obtain the cardio-accelerator effects described by Yung for *Mya*, *Solen*, and *Anadonta* on stimulation of the visceral ganglion.

Mya is one of the best forms for this work, as its shells are sufficiently brittle to be removed piecemeal without much injury to the soft parts. When the shell has been removed on one side, the siphon, and the anterior part of the mantle, and the greater part of the gills cut away, the movements of the auricles can be observed through the ventral side of the kidney. If now the visceral ganglion is stimulated with a weak interrupted current, the pulsations cease, the auricles remaining for some time in diastole. If the strength of the stimulus is comparatively weak, or the stimulation long continued, the beats reappear before the cessation of the stimulation, but with a stronger current complete arrest of the auricles in diastole can be obtained for from one to two minutes. On cessation of the stimulation, the auricles usually beat for some time with a quicker rhythm than the original. But there are several objections to concluding from these reactions that the visceral ganglion sends inhibitory fibres to the auricles. The ventricle and auricles of this mollusc are arrested in diastole on direct stimulation with the interrupted current, and it is possible that the inhibition obtained on stimulation of the visceral ganglion is due to escape of the current through the kidney, and direct to the auricles, as in the specimens of *Mya* or *Tapes* worked on, the base of the auricles is only about 2 cm. distant from the ganglion. The interference with the rhythm may further be due to the pressure on the heart from the contraction of the kidney, and the musculature of the anterior and dorsal part of the mantle, as the ganglion sends motor fibres to these structures. That the inhibition is not caused by escape of the current is shown by the fact that it requires greater strength of the stimulus to produce the standstill when applied directly to the auricles, than when applied to the ganglion. The inhibition of the auricles can also be obtained

reflexly by stimulation of the nerves to the siphon. These siphonal nerves are comparatively stout, and can be isolated for experimental purposes for a distance of 3 to 5 cm. from the ganglion. There is no chance of escape of the current to the auricles, when the weak interrupted current is applied to the isolated siphonal nerve 4 to 5 cm. from the visceral ganglion. The inhibition of the auricles obtained reflexly by stimulation of the siphonal nerve is similar to that obtained by direct stimulation of the ganglion, but complete arrest of the rhythm can usually be maintained for a longer time when the ganglion is stimulated. To observe the ventricle, it is necessary to remove the body-wall and pericardium dorsal to the heart. If this is done, and the visceral ganglion or the siphonal nerve is stimulated as before, the effect on the ventricle is seen to be identical with that on the auricles, the ventricle ceases to beat and remains in a collapsed condition during the stimulation. This interference with the ventricular rhythm is not due to variations in pressure, either intra- or extra-cardial, for the ventricle is empty, and in connection with the auricles, the aorta, and the hind gut only, so that no pressure can be brought to bear on it by the contractions of adjacent parts. The inhibition of the ventricle is obtained after severing the aortæ and the rectum at either end of the heart, leaving only the auricular connections intact, so that there can be no question but that the change in the ventricular rhythm following the stimulation of the visceral ganglion is due to nervous influence on the ventricle reaching it through the auricles.

The foregoing account applies in the main to the rest of these lamellibranchs, particularly *Tapes*, *Venus*, and *Pecten*. The heart and its nervous connections in *Tapes* and *Venus* were prepared for graphic registration by taking the animals out of the shells, care being taken not to injure the region of the heart, removing the greater part of the foot, the siphon, and the gills, and fixing the rest of the animal to a small board in such a manner that the heart and the visceral ganglion with its nervous connections are accessible. The dorsal pericardium is removed, the board supported by a clamp in a vertical position, and a glass or platinum hook passed into the ventricle and connected with a light recording lever. Usually the ventricle was completely severed from the aortæ and the rectum, leaving it in connection with the auricles only. In this way the weight of the ventricle and the lever has to be supported by the relatively delicate auricles. In the case of *Tapes*, even the lightest

recording lever practicable was too heavy to be lifted by the contraction-force of the auricles. The auricles continue to beat, but do not shorten perceptibly in the direction of the pull of the lever. The excursion of the lever shows, therefore, the ventricular contractions only. In *Venus*, the auricles are strong enough to lift the lever. The myograms from the heart preparation of this animal become in consequence very irregular, as under the conditions for the experiments the co-ordination between the two auricles, as well as between the auricles and the ventricle, is not maintained. A tracing from the ventricle of *Tapes* on stimulation of the visceral ganglion under these conditions is given in Fig. 1.

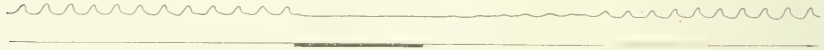


FIGURE 1. — Tracing from the ventricle of the clam (Tapes), showing inhibition of the rhythm on stimulation of the visceral ganglion with the interrupted current.

We may, therefore, conclude that the auricles and the ventricle of these lamellibranchs are supplied with inhibitory nerves from the visceral ganglion or ganglia, and that this cardio-inhibitory mechanism can be excited reflexly by the stimulation of the siphonal nerves No definite accelerator effects on the heart were observed on stimulation of the visceral ganglion either directly or indirectly through the siphonal nerves. The initial inhibition may, especially in *Mya*, be followed by acceleration even when the stimulation is continued, but the very opposite effects, or a continuation of the inhibition for some time after the cessation of the stimulation is also obtained, or the rhythm following the stimulation may be slower than that preceding.

In *Mya*, *Tapes*, and *Venus*, experiments were performed with the view of determining the exact course of the fibres from the visceral ganglion to the auricles and the ventricle. After severing the nerves (in *Mya*) that are given off from the dorsal side of the visceral ganglion, the inhibition is still obtained on stimulation of the ganglion or the siphonal nerve, showing that the fibres do not reach the heart by these nerves. But after severing the nerve plexus on the dorsal surface of the kidney, or the cerebro-visceral commissures close to the ganglion, the stimulation of the ganglion or the siphonal nerve has no further effect on the heart. It is, therefore, evident that the heart is innervated through the renal nerves.

It has already been stated that the inhibition of the auricles, as

well as the ventricle, is obtained after the dorsal pericardium has been removed, and the ventricle severed from the aortæ and the rectum. If, on the other hand, the auricles are severed along their attachment to the afferent sinuses, leaving the heart in connection with the aortæ and the rectum, the stimulation of the ganglion or the reno-cardiac nerves has no effect on the rhythm. This goes to show that no fibres enter the heart along the aortæ or the rectum.

The experiments on the influence of the cardiac nerves on the heart of *Mytilus* lead to no conclusive results. This applies both to the larger species on the Pacific coast and to the smaller one on the Atlantic. The shells of this mollusc are so hard and tough that they cannot be removed without great injury to the soft parts, particularly in the region of the heart. The shells had to be pried apart sufficiently to introduce a scalpel to sever the posterior adductor muscles of the shells, the byssus, and the foot on one side, in order to remove one of the shells. The posterior adductor muscles of the byssus and the foot penetrate the visceral envelope close to the base of the auricles, and the wall of the body cavity, which in this region is at the same time the lateral wall of the pericardial cavity, is closely attached to these muscles. In fact, I never succeeded in severing the muscles from their attachment to the shell, under the condition that it had to be done, without tearing the pericardium, and at the same time causing more or less injury to the auricle of the same side. The lateral walls of the pericardial cavity are partly transparent, so that when one of the shells is removed, the movements of the ventricle can be observed without further dissection. When either of the visceral ganglia in a specimen thus prepared is stimulated, the usual effect on the heart is acceleration of the rhythm. But the stimulation produces, at the same time, contractions in the kidney and the dorsal mantle, and the variation in the rhythm may be caused by the change in pressure on the heart which is brought about by these contractions. At no time did I obtain distinct inhibition of the cardiac rhythm on stimulation of the visceral ganglia, or any of the nerves given off by them. It does not seem probable that cardio-inhibitory fibres should be present in clams, but not in mussels. Possibly the injury to the pericardium and the auricles attendant upon removing the shell in *Mytilus* severs the nervous connections between the heart and the ganglia.

The Chitons.

The chitons offer almost insurmountable difficulties in obtaining workable preparations of the heart and the cardiac nerves, because of the habit of these animals of rolling up into a ball and remaining in that extreme contracted state as long as handled or irritated. For the experiments to determine the function and course of the cardiac nerves, the giant *Cryptochiton* was used almost exclusively, because of its size, the isolation of the nerve cords being attended with greater difficulty in the smaller *Ischnochiton*. Two methods of preparing the animals for the experiments were tried. The animal was placed on its dorsal side on a board, and left undisturbed until completely relaxed, which usually took place in twenty to thirty minutes, and then it was quickly secured to the board by nails driven through the mantle into the board. In this manner the animal was prevented from doubling up, but the contraction of the body musculature was still great and the viscera, in consequence, under so great pressure that they were squeezed out through the smallest slit in the body wall. With the animal in this position, a median incision was made in the foot from its anterior to its posterior end, exposing the viscera. The force of contraction of the divided foot was still so great that the halves could not be bent to the sides, but the median portion of each half had to be cut away to allow removal of the intestinal mass and the hind gut. By a slit in the ventral wall of the pericardium, the heart was exposed to view. The peri-œsophageal nerve ring and the pallio-visceral nerve cords were now exposed and stimulated with the following results. If the heart was beating rhythmically, the stimulation usually produced a slight acceleration of the rhythm; but this acceleration was neither very marked nor long continued; it usually ceased in twenty to thirty seconds, however long the stimulation was kept up. The effects were the same whether the œsophageal nerve ring or the pallio-visceral cords were stimulated with the interrupted current, but preparations were frequently met with in which the stimulation of these nerve cords did not seem to affect the cardiac rhythm. If the heart is quiescent, the stimulation elicits a few beats at its beginning, or starts a rhythmic series of beats that may continue even after cessation of the stimulation. No slowing or arrest of the rhythm was observed from the stimulation; if the heart was affected, it was invariably in the way of acceleration. Of course, it should be borne in mind that any change in the force or magnitude of the

auricular or the ventricular beats could not readily be determined with certainty with the method of direct observation, and that owing to the very slow rhythm of the exposed heart (4 to 7 per minute), a slight decrease in the rate might easily escape the attention. The auricles and the ventricles were empty and collapsed, so that the augmentation of the rhythm could not have been produced by increased intracardial pressure, brought about indirectly by the contraction of the musculature around the gills and the foot and the dorsum. But this method of preparation destroys the pedal nerve cords, as well as all nervous elements that may pass to the ventricle along the hind gut or in the ventral wall of the pericardium. In order to determine whether the stimulation of these nerve cords produces the same effect on the heart when the pedal cords, the viscera, and the pericardium remain intact, the animal was nailed to the board, ventral side down, and the œsophageal nerve ring exposed from the dorsal side. The hindmost shell, together with a small part of the dorsum above the heart, were removed to allow the movements of the heart to be observed. Although the shell was removed as carefully as possible, I never succeeded in doing so without slight injury to the posterior end of the ventricle, because the ventricle is attached directly to the internal lining of the shell. From the pressure on the viscera, due to the strong contraction of the foot and the dorsum, the cardiac apparatus is displaced posteriorly in the opening made by the removal of the shell. The same is true of the buccal mass, and the anterior portion of the viscera, which tend to protrude through the opening made by the dissection for the œsophageal nerve ring. But despite the heart being thus pushed outward, and subjected to abnormally great pressure from the viscera, the ventricular rhythm continues fairly regular, with a rate of from four to six beats per minute. Stimulation of the exposed œsophageal nerve cord in this preparation produces increased pressure on the viscera from the additional contraction of the body muscles, and at the same time augmentation of the rate of the heart beats. The acceleration of the rhythm is usually more marked on stimulation of the œsophageal nerve cord than on stimulation of the pallio-visceral cords, probably because in the former case the nervous elements to both auricles are stimulated simultaneously, which is not done by stimulation of the right or the left pallio-visceral cord. At no time did the stimulation produce inhibition of the rhythm. But with the animal prepared in this manner, it is possible that the augmentation of the rhythm is due,

not to the action of augmentor nerves on the heart, but to the increased pressure in the cavities of the heart as well as to the increased external pressure and displacement. With the first method of preparation, these stimuli could not be operative, and as the results are the same with both methods of preparation, it seems very probable that *the heart of the chitons is provided with accelerator nerves, reaching the heart from the pallio-visceral nerve cords.* While I failed to find any evidence of the presence of cardio-inhibitory nerves, we may not conclude that these molluscs have no cardio-inhibitory nerves, especially in view of the difficulties in obtaining preparations with the heart and possible nervous connections intact. I could not determine whether the augmentation of the ventricular rhythm was due to nervous action on the ventricular muscle, or to the increased rhythm of the auricles. The former alternative is probably the correct explanation, because the acceleration of the ventricular rhythm was obtained even in preparations in which the auricles and the ventricle did not beat synchronously.

The Prosobranchs.

The abalone and the limpet offer less difficulties than the lamellibranchs and the chitons in experimenting on the function of the cardiac nerves, and one does not have to rely on direct observation entirely, but can record the influence of the nerves on the heart more accurately by the ordinary graphic method. With *Haliotis* I proceeded in the following manner. The shell was removed carefully so as not to injure the gills and the viscera on the left side. The pallio-visceral connectives were severed from their connections with the pleuro-pedal cords and dissected free for a distance of 3 to 4 cm., as they pass lateral and posterior in the roof of the visceral cavity. The gills and the whole visceral mass were separated from the foot. The anterior part of the gills, together with the mantle, were then cut away, the dorso-lateral wall of the pericardial cavity slit open, and the liver and intestinal mass posterior to the pericardial cavity removed. The preparation that now remained, consisting of the pallio-visceral connectives, the floor of the posterior end of the gill chamber, the heart and part of the kidney and the hind gut, was firmly fixed to a board of convenient size in such a way that contraction of any of the adjacent tissue would not in any way affect the position or movements of the heart. After some trials, I nearly always succeeded in this dissection, and fixing of the preparation without in

jury to the nervous connection with heart. The cardiac rhythm can now be studied directly, or the board fastened vertically by means of a clamp, a hook from a light recording lever attached to the ventricle, and the contractions recorded on the myograph. The results obtained on these preparations were compared with the reactions of the heart to stimulation of the œsophageal nervous complex or the pallio-visceral commissures, when the nervous system and the entire visceral mass were left intact, the movements of the heart being observed through the slightly transparent dorso-lateral wall of the pericardial cavity.

In *Lucapina* the shell and the mantle were removed and the dorsum, with the viscera attached, separated from the foot. The pallio-intestinal and the intestino-visceral connectives run in the connective tissue close to the dorsum, and must be dissected from the ventral

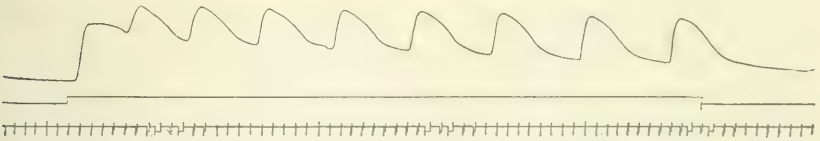


FIGURE 2. — Tracing from the ventricle of Haliotis on stimulation of the pleuro-visceral nerves with the interrupted current. Ventricle quiescent prior to the stimulation.

side. After freeing the commissures, the anterior part of dorsum, together with the liver, intestines and reproductive glands, and the dorsal pericardium, were cut away, and the preparation fixed to the board in a manner similar to that of the abalone preparation.

Before going into the more detailed description, it may be stated that *both in the abalone and the limpet stimulation of the œsophageal nervous complex or the pallio-visceral connectives, all the visceral organs being intact, or stimulation of the commissures in the above described preparations has accelerator effects on both the auricles and the ventricle; if the heart is quiescent, the stimulation produces a series of beats, if in a state of rhythmical pulsations, the rhythm is augmented.* In *Haliotis* the accelerator effect on the ventricle is just as marked after the ventricle is separated from the auricle, and when the graphic method was employed the auriculo-ventricular connections were usually severed, that the lever might not be affected by the movements of the auricles. The hook was fastened to the ventricle near the aortic end. Fig. 2 is a typical record from the quiescent ventricle on stimulation of one of the commissures with the weak interrupted current. The effect is

greatest at the beginning of the stimulation, the diastole being then so much shortened that there is a partial fusion of the beats. In fresh and vigorous preparations the stimulation ceases to be effective after one or two minutes, the gradually increasing diastoles passing into quiescence of the ventricle. The weaker the stimulus the sooner it ceases to be effective. In more fatigued preparations the heart ceases to be influenced by the stimulation much sooner, until towards the end of an experiment only one or two beats at the beginning of the stimulation are produced. When a weak interrupted current is used the stimulation usually affects the heart only during the first ten to twenty seconds, even in fresh preparations. Whether this rapid failure of the stimulation is due to the fatigue of the muscle, or to failure of the nervous mechanism, must be undecided for the present. I very

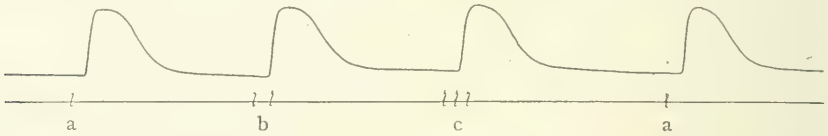


FIGURE 3. — Quiescent ventricle of Haliotis. The pleuro-visceral nerves stimulated with single induced shocks. Showing a relative refractory condition of the nerve-heart preparation during systole.

seldom succeeded in obtaining preparations from the abalone in which the ventricle continued in rhythmical contractions for any length of time when subjected to the experimental conditions. This was probably due to the considerable time required by the dissection, as well as to the unavoidable injuries sustained by the heart in being prepared.

In fresh and vigorous preparations, the resting ventricle responds with single beats to single induction shocks of not very great intensity applied to the pleuro-visceral connectives. As the preparation fatigues, two, three, or even four shocks following each other in rapid succession, are required to produce a beat. When a single induced shock is effective, any additional number of shocks applied to the commissures during the systole or the beginning of the diastole are without visible effect on the heart. This is typically illustrated in Fig. 3. The contraction produced by the single shock at *a* is of the same strength and duration as the contraction produced by two shocks at *b*, or by three shocks at *c*, the intensity of the shocks being the same in each case. That this is not due to failure of the nervous mechanism seems to be shown by the accumulative effects which are obtained with exceedingly weak stimuli, or in the case of fatigued preparations (Fig. 4).

A strong constant current (2 to 3 Edison-Lalande cells) applied to the commissures gives a beat on the make, if the kathode is peripheral, that is, nearest the heart, and a break beat, if the current is ascending in the nerve. In rare instances I have observed two or three beats on the make of the descending current in the fresh and vigorous preparations.

We have seen that single induction shocks of an intensity that is slightly painful to the tongue applied to the commissures at a distance

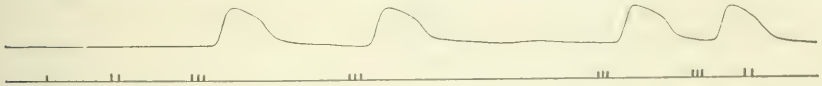


FIGURE 4. — Ventricle of Haliotis, fatigued. The pleuro-visceral nerves stimulated with weak induced shocks. Summation.

of 5 to 6 cm. from the heart are able to produce beats in the resting ventricle. And from the effectiveness of the interrupted current, one would expect that, within limits, the more rapid the succession of the single shocks, the greater would be the number of beats produced in a given time. This very reaction is shown in Fig. 5, where the induced shocks applied to the nerve at the rate of one per second produce a greater pulse rate than the shocks of the same intensity applied at the rate of one every third or fourth second; but while experimenting along this line records were frequently obtained that

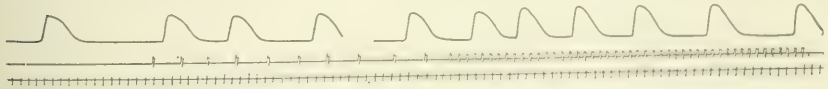


FIGURE 5. — Record from the ventricle of Haliotis, beating very slowly. The pleuro-visceral nerves stimulated with weak induced shocks. Time in seconds.

showed the very reverse reaction and suggested at first sight a possible inhibitory effect on the heart from the stimulation of the commissures. Typical tracings of this type are reproduced in Fig. 6, *a*, *b*, and *c*. It will be seen from these records that if the shocks are applied to the nerves at the rate of one every eighth or tenth second, each stimulus produces a beat of the ventricle, but if the rate is increased to one shock in every or every other second, the ventricle remains in a continuous diastole; if then the rate of the stimulation is again changed to one shock in every eighth or tenth second, each shock produces a ventricular beat. The tracings similar to those reproduced in Fig. 6, *b* and *c*, give the clue to the interpretation of these singular results. In

the preparations in which the ventricle was quiescent, this prolonged diastole was usually obtained by the rapid series of shocks; but if the ventricles were beating spontaneously, contractions would invariably appear during such stimulations (Fig. 6, *b* and *c*), and *the rate of these contractions corresponded fairly closely to the original rhythm.* This will be seen by comparing tracings *c* and *d*, which are from the same preparation. Tracing *d* gives the rate of the spontaneous beats, and although the rhythm is slow and quite irregular, the three beats of the ventricle during the rapid stimulation of the nerve in tracing *c*, appear nevertheless to be in the series of the original rhythm. If this is the correct interpretation, it is evident that the prolonged diastole during the rapid stimulation is not produced by inhibitory nervous impulses,

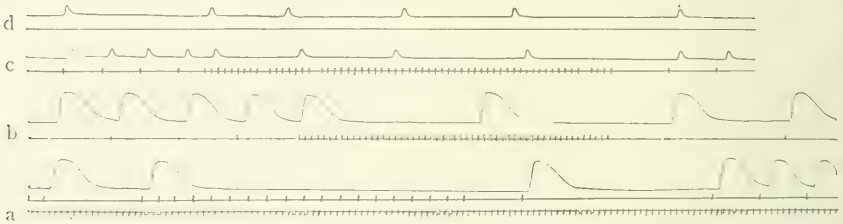


FIGURE 6. — Haliotis, *a*, *b*, tracings from resting ventricle on stimulation of the pleuro-visceral nerves with single induced shocks. *c*, tracing from slowly beating ventricle on similar stimulation of the nerves. *d*, record showing the spontaneous rhythm of the same ventricle as tracing *c*. Apparent diminution in the rate of the beats on increasing the rate of stimulation of the pleuro-visceral nerves. Time in seconds.

nor is it caused by the fatigue of the heart; but it is the result of failure of the nervous mechanism, be this a reduced excitability of the nerves, or a condition of fatigue of the nerve endings. If the stimulus fails to produce a nervous impulse, or if the impulse is started, but does not reach the ventricle, it is obvious that the quiescent ventricle will remain in that condition and that the pulsating ventricle will give contractions corresponding to those of the original rhythm. In fact, the same results are obtained in the fatigued preparations with the interrupted current, which in such cases produces only a few beats at the beginning of its application to the commissures. And the further fact that in fresh and vigorous preparations, increasing the rate of the stimulation increases instead of decreases the rate of the beats, points to the same conclusion, namely, that the apparent inhibition of the beats by the rapid series of induction shocks is really caused by the failure of the augmentor or motor nervous impulses to reach the ventricular muscle.

The application of the interrupted current to the commissures is followed by what appears to be an increased tonus of the ventricular muscle. The greater part of this rise of the base line is probably due to the contraction of the muscular tissue about the rectum and the gut to which the ventricle is attached. These parts are also supplied with motor nerves through the pallio-visceral commissures, and it was extremely difficult to fix the preparation to the board so that the contractions of these parts would not in any way be communicated to the ventricle, and at the same time leave intact the nervous connection with heart. With some preparations I succeeded better than with others, and in such cases the apparent tonus increase is much less in evidence.

The response of the ventricle to stimulation of the commissures is not impaired by severing the auricles from the ventricle, or by severing the gut anterior to the ventricle; but if the auricular and the aortic connections are severed, the stimulation of the commissures has no effect on the ventricle. This goes to show that *the augmentor fibres enter the ventricle by the posterior or aortic end of the ventricle*. If the ventricle is severed from its connection with the pericardium by sectioning the gut anterior to the ventricle, and the aorta and the gut at the posterior end, leaving the ventricle in connection with the auricles only, care being taken not to injure the ventricle, the ventricle still appears to respond slightly to the stimulation of the commissures. This may be due to nerves that enter the ventricle through the auricular walls, or it may simply be a continuation of the contraction started in the auricles by the stimulation. The latter is probably the correct explanation, because when the excitability of the heart is reduced, the auricular contractions cease at the auriculo-ventricular junctions, and the ventricle remains quiescent, except for the displacements by the contraction of the auricles.

Stimulation of the commissures produces augmentation of the rhythm in the auricles, just as in the ventricle. No inhibitory effects were observed on either the auricles or the ventricle. If the stimulation had any effect on the heart, it was in the line of acceleration.

Stimulation of the pleuro-intestinal or the intestino-visceral commissures in the limpet has the same effect on the heart as the stimulation of the pleuro-visceral connectives in the abalone. The *Lucapina* material was less abundant, so that the experiments on this mollusc were not so extensive as in the case of *Haliotis*. The *Lucapina* preparation offers one advantage over that of the abalone in the more

constant rhythm of the heart when subjected to the experimental conditions. In the Lucapina preparations with the heart in the state of rest, the ventricular beats produced by the stimulation of the commissures usually follow each other with increasing magnitude of contraction in a "staircase" fashion. This can also be made out on the first few beats following the stimulation in the preparations in which the heart is beating rhythmically, for in these preparations not only is the duration of the diastole decreased, but the magnitude of the contraction is increased (Fig. 7).

The acceleration is more marked on the auricles than on the ventricle; and it is the same on stimulation of either the pleuro-intestinal or the intestino-visceral division of the commissures.

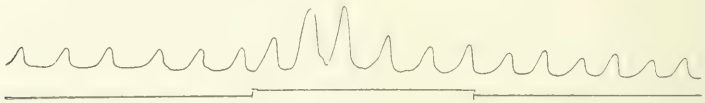


FIGURE 7. — Tracing from the ventricle of the limpet (*Lucapina*) on stimulation of the pleuro-visceral nerves with the interrupted current. Showing augmentation.

The effect on the heart of stimulation of the commissures with the constant current is the same as in the abalone, with the exception that in three preparations there appeared to be partial inhibition of cardiac rhythm during the passage of the strong constant current through one or both of the intestino-visceral nerves. The electrodes were in every case applied to the isolated commissures at a point more than 4 cm. distant from the heart, so that the effect could not have been produced by escape of the current directly to the heart. On the other hand, the failure to obtain any evidence of inhibitory influence on the heart, on stimulation of the commissures with the induced current of varying intensities, seem to militate against interpreting the reactions as due to nervous inhibition. Further studies are required on this point.

In reviewing the literature on the innervation of the heart in the prosobranch molluscs,[1] it was pointed out that the nerves to the auricles were described by several observers as coming from the gill ganglia and not from the visceral ganglia. Illingworth makes that statement expressly for *Lucapina crenulata*. But stimulation of the branchial ganglia, or either of the two nerves that pass in the posterior direction from each ganglion, has no effect either on the auricles

[1] CARLSON: Biological bulletin, 1905, viii, p. 123.

or the ventricle, while the stimulation of either of the commissures after severing the connectives between the intestinal and the gill ganglia augments the rhythm of both auricles. It is, therefore, evident that the auricles as well as the ventricle are supplied with nerves from the visceral ganglia.

The motor nerves to the ventricle appear to enter by the aortic end, just as in *Haliotis*, from the fact that after severing the auricular connections and the rectum, stimulation of the commissures still produces augmentation of the ventricular rhythm; but after sectioning the auricles and the aortic sinus, leaving the rectum intact, the stimulation has no further effect on the ventricle.

Of the large group of monotocardic prosobranchs, only two representatives of sufficient size for the work were available, namely, the large *Natica* on the Pacific coast, and the still larger *Sycotypus* obtained at Woods Holl, Mass. The *Sycotypus* material was the more abundant. This gastropod is not only larger than *Natica*, but it can be removed from its shell with less difficulty. The bulk of the work was done on this form. The great size and strength of the heart and the relative ease with which it may be isolated, together with its nervous connections for the purpose of experiments, make *Sycotypus* one of the most favorable molluscs for this work. There appears to be no difference between the physiology of the cardiac nerves in the diotocardic and the monotocardic prosobranchs.

When *Sycotypus* or *Natica* is removed from the shell and the heart exposed by removal of the pericardium dorsal to the heart, the rhythm is very slow, especially in *Natica*. In the latter animal the heart frequently remains quiescent for thirty minutes or more before a slow rhythm appears, and sometimes no spontaneous rhythm appears at all. The heart of *Sycotypus* exposed in this manner usually pulsates with a rate of 5 to 8 per minute. The quiescence or slow rhythm is not due to inhibitory influences from the brain or the visceral ganglia, these nerve centres being stimulated by the severe injuries to the animal on removing it from the shell and exposing the heart, because severing the nervous connections to the heart does not augment the rhythm. Nor is it due to excessive pressure of the blood in the heart, for although the animal is in extreme contraction, the heart is nevertheless empty, as the removal of the animal from the shell causes profuse bleeding. Lesion of the aortic sinus has, furthermore, no effect on the rhythm.

When in these preparations the pleuro-visceral commissures are

stimulated with the interrupted current, the rhythm of the beating heart is augmented both in rate and in amplitude of contractions, and if the heart is quiescent, the stimulation produces a series of contractions both in the auricle and the ventricle. These reactions are produced by stimulating either one of the commissures, as well as by stimulating the visceral ganglia directly. They are furthermore obtained on stimulating the pleural ganglia, either directly or by stimulation of afferent nerves. When the nerve from the right visceral ganglion to the ganglion on the aortic junction is stimulated with the interrupted current, the augmentation of the ventricular rhythm approaches tetanus.

After lesion of the heart at the auriculo-ventricular junction, and allowing the stimulating effects of the section to subside, stimulating the commissures or the visceral ganglia still produces these accelerator effects both on the auricle and the ventricle. It is evident, therefore, that nerves enter the heart both at the auricular and at the aortic ends. When the heart has been severed at the aortic junction, the stimulation of the commissures or ganglia has no appreciable effect on the ventricle; but its effect on the auricle is not diminished. If both auricle and ventricle are quiescent, the relative independence of the ventricle is shown by the fact that the stimulation starts a series of contractions in the auricle, but the ventricle remains at rest. It is, therefore, probable that the nervous supply to the ventricle reaches the ventricle solely by the aortic end.

No inhibitory effects either on the auricle or the ventricle were obtained by stimulation of these nervous elements or by stimulating afferent fibres to the brain, the pleuro-visceral commissures remaining intact.

In *Natica* the stimulation of the branchial ganglion, after severing its connection with the left visceral ganglion, has no effect on the heart, and in *Sycotypus*, in which the commissure does not connect with the branchial ganglion, the stimulation is similarly without influence. This makes it practically certain that no inhibitory or accelerator fibres pass to the heart from this ganglion.

There might be one objection to concluding from these experiments that the auricles and ventricle of these molluscs are provided with motor nerves. Except in *Sycotypus*, I found it experimentally impossible to so isolate the nerves in the living tissue that they could be stimulated apart from their connections with the motor nervous supply to parts adjacent to the heart. In the limpet and the abalone

we have to be content with working with the intestino-visceral or the pleuro-visceral connectives, which contain motor fibres to the kidney, the floor of the pallial cavity, the rectum, and the muscular parts of the pericardium. Now, may not the beats of the resting heart, as well as the quickened rhythm of the pulsating heart that follow the stimulation of these commissures, simply be caused by the mechanical stimuli from the displacement of the heart due to the contraction of adjacent structures? In the fresh and vigorous preparations the heart is very sensitive to mechanical stimuli, which act in the line of acceleration and not of inhibition. But that mechanical stimuli are not material in producing the augmentation of the heart rhythm following the stimulation of the nerves, is shown by the fact that the acceleration is just as marked in the preparations which are fixed so that very slight or no displacement of the heart is produced by the contraction of adjacent structures, as in the preparation with which no such precautions are taken. In fatigued preparations the excitability of the ventricle to mechanical stimuli are so much reduced that the ventricle may be pulled about and displaced much more than takes place when the contraction of the gills, the rectum, the kidney, and the pericardium have full sway, without giving rise to a beat; yet in such preparations stimulation of the nerves is followed by a series of ventricular contractions. There is no more displacement of the ventricle that has been separated from the auricles and the rectum than of the ventricle separated from the auricles and the aorta and the gut at the posterior end of the heart, and yet the stimulation of the nerves is followed by beats in the former, but not in the latter, evidently because the ventricular nerve enters by the aortic end. Variation in the intracardiac pressure does not figure, because the acceleration is just as evident in the empty and collapsed as in the filled heart. The evidence seems, therefore, conclusive that the auricles and the ventricle of these prosobranchs are provided with accelerator nerves.

The Tectibranchs.

The accelerator effects on the heart of *Aplysia* of stimulation of the pleural ganglia or the pleuro-visceral commissures have been observed by Dogiel (1877), Ransom (1884), Schoenlein (1894), and lately proved more conclusively by Bottazzi and Enriques (1901). None but motor or accelerator effects were obtained by these observers, and Bottazzi and Enriques conclude that there is no inhibitory

nervous mechanism to the heart of this mollusc. In a recent paper Straub comes to the conclusion that the heart of *Aplysia* is not provided with regulative nerves. Straub's negative results are almost certainly due to faulty technique.[1]

My own work was done on *Aplysia californica*, *Bulla globosa*, and *Pleurobranchæa californica*. The previous investigators appear to have relied solely on direct observation. In *Aplysia* I have succeeded in obtaining preparations of the ventricle and the ventricular nerve which lend themselves admirably to the ordinary graphic registration, and have, therefore, been able to study the influence of the nerves on the heart in greater detail. The main results may be stated at outset, namely, that *the auricle and the ventricle of Aplysia, Bulla, and Pleurobranchæa are supplied with accelerator nerves, and that there is no evidence of the presence of an inhibitory nervous mechanism.*

The removal of the dorsal body wall and the pericardium dorsal to the heart in *Aplysia* does not interfere with the cardiac nerves. But this operation, together with the dissection required to expose the pleural ganglia and the visceral nerves, causes so profuse bleeding that the heart is soon rendered empty and collapsed. Rhythmical pulsation is kept up for an hour or so after the operation. With the heart intact, stimulation of the pleural ganglia or the visceral nerves with a weak interrupted current produces great augmentation of the rhythm in the pulsating heart and starts a series of beats in the resting heart. The acceleration appears to involve both the auricle and the ventricle. When the heart is severed from the aortic sinus, the stimulation will still cause augmentation of both the auricular and the ventricular rhythm, but the acceleration is most marked in the auricle. If the heart is severed at the auriculo-ventricular junction, the stimulation produces acceleration both in the auricle and the ventricle as before. It cannot be determined whether augmentation of the auricular rhythm can be obtained after severing the auricle at its base, because that operation involves so great injury to the auricle that its musculature goes into prolonged contraction. It is evident from these experiments that accelerator nerves enter the ventricle along the aortic sinus and the auricle at its base. Accelerator nerves may also reach the ventricle through the walls of the auricle; but I

[1] Dogiel: Archiv für mikroskopische Anatomie, 1877, xiv, p. 59; Ransom: Journal of physiology, 1884, v, p. 261; Schoenlein: Zeitschrift für Biologie, 1894, xviii, p. 187; Bottazzi and Enriques: Archives italiennes de biologie, 1901, xxxiv, p. 111; Straub: Archiv für die gesammte Physiologie, 1904, ciii, p. 429.

am rather inclined to interpret the accelerator effects observed in the ventricle after separation from the aortic sinus as due to the contraction waves of the auricle passing on to the ventricle.

For graphic registration, the pleuro-visceral commissures were isolated down to the visceral ganglion, and the nerves to the osphradium, the gill, and the posterior dorsum severed from the ganglion, leaving only the visceral nerve proper intact. A silk ligature was secured to the auriculo-ventricular junction, and the auricle severed from the ventricle. The ventricle, together with the nerves, the aortic sinus, and part of the ventral pericardium was now removed from the rest of the body, and as much of the tissue around the aortic sinus cut away as could safely be removed without severing the ventricular nerves. The ventricle was suspended by means of a fine



FIGURE 8. — Tracing from the ventricle of Aplysia on stimulation of the pleuro-visceral commissures. Ventricle severed from the auricle. Augmentation of the rhythm.

platinum hook passed through the aortic sinus, and by the silk ligature at the auricular end. The tissue left attached to the aortic sinus was supported independently in such a way that its contraction would least or not at all affect the ventricle. Some of the ventricles prepared and suspended in this manner continued in a strong and regular rhythm for ten to twenty minutes, but in the majority of the preparations the ventricle remained quiescent, undoubtedly owing to injuries received in the dissection.

In fresh and vigorous preparations the application of a weak interrupted current to the pleuro-visceral connectives produces so great augmentation of the ventricular rhythm that the beats fuse almost to a tetanus without any marked increase in the tonus or the strength of the contractions (Fig. 8). In more fatigued preparations, or if an extremely weak stimulus is used, the shortening of the diastole and the diastolic pause is much less marked. If the ventricle is quiescent, the stimulation of the commissures starts a series of beats of gradually decreasing frequency. In fresh preparations the rhythm started by the stimulation, usually continues for some little time after the cessation of the stimulation, but in more fatigued ventricles the beats cease during the stimulation, if this is kept up for a minute or two,

and in every case at the cessation of the stimulation. In the resting ventricles, the systole of the initial beat following the stimulation of the nerves with the interrupted current is much more prolonged than that of the subsequent beats. In some cases the time occupied by this beat is as long as that of two or three of the subsequent beats, especially if a strong interrupted current is employed. But in every case it appears to be a single beat rather than a fusion of two or more beats.

Single induction shocks of moderate intensity applied to the commissures do not always affect the ventricle. In the very fresh and vigorous preparations, however, the single shock usually produces a beat in the quiescent ventricle or a shortening of the diastole in the pulsating ventricle. But if the induced shock is applied to the "visceral nerve" instead of to the commissures, it nearly always produces a beat, even when applied during systole or at the beginning of diastole. In more fatigued preparations this is not the case, however, the shocks applied to the nerve during systole and diastole, as well as at the beginning of the diastolic pause, are without effect on the ventricle, probably owing to the reduced excitability of the heart in these phases of contraction. The beats produced by the single shocks, applied to the nerve during the ventricular systole or diastole, are usually sub-maximal (Fig. 9); but tracings were also obtained showing this extra contraction to be of normal or maximal height. When an induced shock of very great strength is employed, it appears to affect the heart, even when applied to the commissures or the pleural ganglia at the very beginning of the ventricular systole. The effect does not appear in the strength of that beat, but as an extra contraction on the succeeding diastole. The rate of propagation of the motor nervous impulse in the tectibranch molluscs is only $\frac{3}{4}$ m. per second; but still the nervous impulse started by the stimulus must have reached the

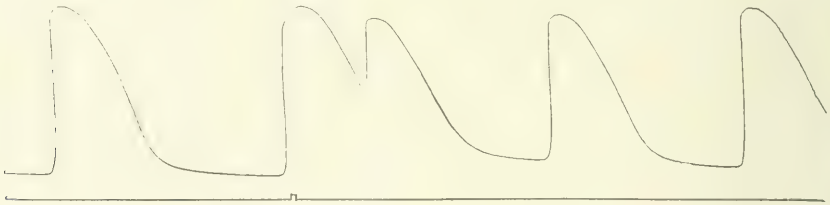


FIGURE 9. — Ventricle of Aplysia. The ventricular nerve stimulated with a single induced shock at the beginning of systole. Showing excitability of the nervous mechanism during systole.

heart long before the appearance of the extra contraction. Probably the commissures are injured by an induced shock of such great strength so that a series of nervous impulses, instead of a single impulse, is produced.

The accumulative effects on the heart of the stimulation of the commissures can be shown by using induced shocks of weak strength. These shocks fail to affect the ventricle singly, but when following each other at the rate of two or more per second, a ventricular beat is produced.

The constant current applied to the commissures produces make and break beats in the resting ventricle; and when the current from

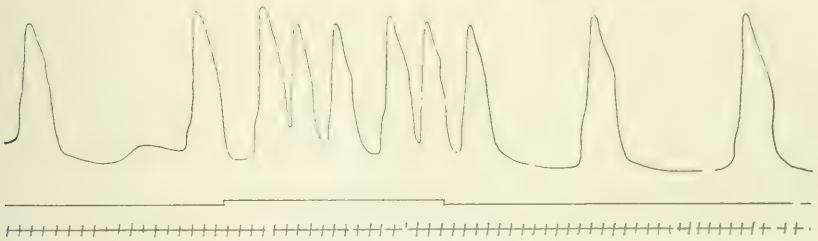


FIGURE 10. — Ventricle of Aplysia. Augmentation of the rhythm during the passage of the strong constant current through the pleuro-visceral commissures.

3 or 4 cells is used, augmentation of the rhythm is produced during the closure of the circuit, provided the preparation is in good condition (Fig. 10).

The sole function of the cardiac nerves thus appears to be augmentor. Stimulation of the œsophageal nervous complex, the commissures, or the "visceral nerve" produces acceleration in the auricle and the ventricle, whether the heart is pulsating or quiescent. Either there are no cardio-inhibitory nerve fibres in the commissures, or the accelerator fibres are so predominant that when the two antagonistic sets of nerve fibres are stimulated simultaneously the inhibitory effects are completely overshadowed by the accelerator effects. If this is the case, it seemed possible that by inducing a condition of extreme tonus (or tetanus) in the ventricle the inhibitory influence might be more readily brought to light in relaxation or decrease of tonus, especially as this reaction of the heart on stimulation of the cardio-inhibitory nerves is very marked in the cephalopods. A series of experiments were carried out to test this hypothesis. The suspended ventricle was made to complete a circuit, the platinum hook

which supported the ventricle and the silk ligature at the auriculo-ventricular junction serving as poles on the ventricle, the silk thread being moistened with the blood plasma. This circuit, as well as the electrodes by which the commissures were stimulated, were connected with a commutator without cross bars, so that the induced current could be sent directly through the suspended ventricle or through the nerves at will. When a strong interrupted current is sent directly through the ventricle, a strong tonus with a rapid series of diminutive beats is produced, lasting for some time after the cessation of the stimulation, the longer, the stronger the stimulus. When the in-

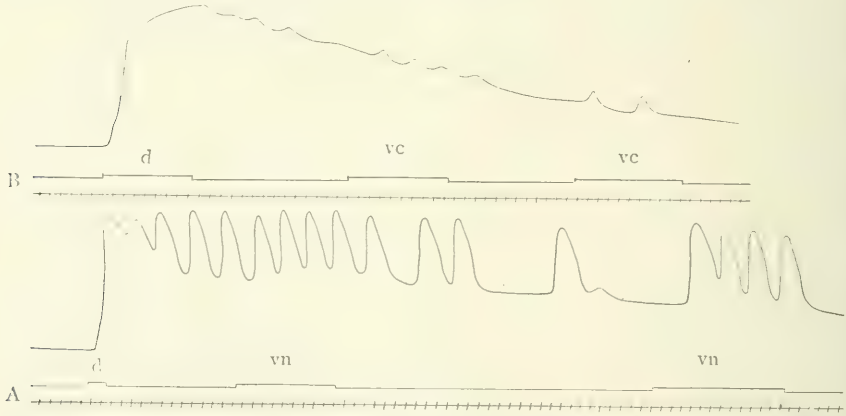


FIGURE 11. — Quiescent ventricle of Aplysia. *d*, direct stimulation of the ventricle with the strong interrupted current. *vn*, stimulation of the ventricular nerve. *vc*, stimulation of the pleuro-visceral commissures. Showing rhythm and further tonus contraction on stimulation of the heart nerves. Time in seconds.

terrupted current is very strong, no beats at all appear on the tetanus or tonus curve. If on the cessation of the direct stimulation of the ventricle, the ventricle being in strong tonus or tetanus, the interrupted current of moderate strength is applied to the commissures, no decrease in the tonus or relaxation of the ventricle is produced under any conditions; but when the stimulation of the nerves affects the ventricle at all, the effects are in the line of acceleration of the beats or further increase in the tonus or tetanus contraction. This is fully illustrated by the tracings reproduced in Fig. 11. In Fig. 11 A, the resting ventricle was stimulated directly for two seconds, with a moderately strong interrupted current which produced a great tonus contraction, together with a rapid series of beats. Yet when the "visceral nerve" is stimulated with a weak interrupted current,

a still further tonus increase is produced, and when the rhythm has ceased, the ventricle remaining quiescent and in strong tonus, the stimulation of the nerve starts a series of beats. The same reactions are brought out in Fig. 11 B. In this case the commissures were stimulated, and the strength of the interrupted current sent directly through the ventricle was very great. The stimulation of the commissures produces diminutive beats on the tonus curve. Thus even this method, which so strikingly demonstrates the inhibitory function of the cardiac nerves in the cephalopods, fails to indicate any inhibitory function of the cardiac nerves in *Aplysia*.

The heart of *Bulla* is so much smaller than that of *Aplysia* that a preparation similar to that of the Aplysia heart for graphic registration could not easily be secured. But the œsophageal nervous complex and the pleuro-visceral commissures were stimulated under all the conditions detailed for *Aplysia*, with almost identical results as regards the effects on the heart. The visceral commissures are readily excited by the induced current, and when they are first stimulated with a weak interrupted current, the heart goes into a more complete tetanus than was ever observed in the Aplysia heart on like stimulation. The single induced shock is also more often efficient. A weak induced shock applied to the commissures produces in the quiescent heart in many cases not only a single beat or two or three beats, but often a long series of rhythmical pulsations.

When the heart is intact, and the movements of the ventricle recorded, a peculiar grouping of the beats sometimes appears in the rhythm produced by stimulation of the commissures with the weak interrupted current, as if there were alternating periods of greater and less excitability of the muscle, apart from the variations in the excitability during each beat. The number of beats in a group is variable, the most usual being three or four. I have no explanation to offer of these peculiar reactions. So far as could be determined, there was nothing in the experimental conditions which might account for them, or for their presence in some preparations and not in others.

It has already been stated that the search for cardio-inhibitory nerves in *Aplysia* was in vain. But in that mollusc the heart cannot be observed without considerable dissection about the heart in removing the dorsum and the dorsal pericardium, and there is a bare possibility that the inhibitory nerves are severed by this dissection. This can be put to the test in *Bulla*, because in this animal the car-

diac rhythm can be observed without previous dissections about the heart. The shell is delicate, and the animal can be taken out of it without being subjected to much injury, and when the shell has been removed the heart can be observed through the transparent dorsal wall of the pericardial cavity. The effect on the heart of stimulation of the commissures or the pleural ganglia under these conditions is the same as when the dorsal pericardium is removed, that is, augmentation. Stimulation of the periœsophageal nervous complex augments the rhythm, provided the visceral connectives are intact; with these severed, the stimulation of these ganglia fails to affect the heart.

I was not able to obtain sufficient *Pleurobranchæa* material to make as extended observations on the function of the cardiac nerves in this species as was done in *Aplysia* and *Bulla;* but the results of the rather limited experiments show that this mollusc falls in line with the two other tectibranchs. Stimulation of the cerebro-pleural ganglion, or of either or both of the pleural-visceral commissures, augments the rhythm of both auricle and ventricle. No definite inhibitory effects on the heart were obtained, either by stimulation of the cerebro-pleural ganglion or the commissures.

From the results in these representatives it is very probable that *cardio-accelerator nerves are present in the entire group of tectibranchs,* but inhibitory nerves to the heart are yet to be discovered.

The Nudibranchs.

Of the several species of the nudibranch group available for the work, only two were of sufficient size and abundance to make the results of the experiments on the cardiac nerves conclusive; these were *Montereina* and *Triopha,*[1] the former being the larger and the better suited of the two. For the experiments on *Montereina,* the animal was fixed to a small board by pins through the edges of the mantle and the foot, and the viscera exposed by removal of the dorsum back to the gill. Generally the foot had also to be cut in two transversely, to relieve the tension on the viscera caused by its extreme contraction. The heart lies posterior and dorsal to the visceral mass, and the pericardium is transparent, so that the movements of the heart can be observed after removing the dorsum; but the pericardium was usually slit open and partially removed, to allow perfectly free

[1] These molluscs have recently been described by Dr. McFarland. Proceedings of the Biological Society of Washington, 1905, xviii, p. 34.

movement of the heart. If these preliminary dissections are done with sufficient care, and without touching the heart with the instruments, the heart continues to beat with a rapid and constant rhythm; but if any part of the heart is handled directly with the forceps, or otherwise injured, it goes into a state of extreme contraction, lasting in some cases as long as five minutes. As the heart begins to relax, rhythmical pulsations reappear.

When the visceral nerve in this preparation is isolated for some distance from the brain, and stimulated with a weak interrupted current, the effect on the heart is augmentation of the rhythm, or the starting of a rhythmical series of beats in the case of the resting heart, just as in the prosobranchs and the tectibranchs. The same effect is obtained by stimulating the brain, provided the visceral nerve is intact. If the heart is severed at the auriculo-ventricular junction, and time given for relaxation of the prolonged contraction of the auricle and the ventricle which is produced by the injury, stimulation of the nerve is followed by the same augmentor effects, both in the auricle and the ventricle. This makes it evident that the accelerator fibres reach the ventricle by the aortic end, probably in the trunk of the branch from the visceral nerve to the ganglion on the aorta. The accelerator fibres to the auricle probably reach that organ in the branch from the visceral nerve to the pericardium and the base of the auricle. When the ventricle is separated from the aortic sinus, but left in connection with the auricle, stimulation of the nerve still produces a slight augmentation of the ventricular beats, which may be due either to the acceleration of the auricular rhythm, or to the influence of a few augmentor fibres that may reach the ventricle through the auricular walls.

While stimulation of the visceral nerve undoubtedly produces augmentation of the cardiac rhythm, these accelerator effects, particularly on the ventricle, are usually not long maintained, even though the stimulation is kept up, and the acceleration is usually followed by a prolonged diastolic pause. This is particularly true when the ventricle is subjected to the tension of the recording lever, instead of lying empty and collapsed in the pericardial cavity. The devices for obtaining direct graphic records from the ventricle of *Montereina* were much the same as those already described for *Aplysia*; but the recording lever had to be very much lighter, as the strength of the ventricle, even in the largest specimens, is relatively small. This almost momentary augmentation of the rhythm, with the subsequent diastolic pause so frequently obtained on stimulation of the visceral

nerve, is illustrated in Figs. 12 and 13. In Fig. 12 the ventricle was quiescent and greatly relaxed when the nerve was stimulated. The rhythm produced by the stimulation is accompanied by great increase in the tonus, and that we have here to do with a real and not an apparent change in the tonus due to the contraction of the tissues to which the aortic sinus is attached is clearly evident on direct obser-

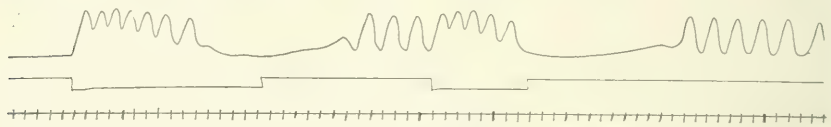


FIGURE 12. — Tracing from the ventricle of the nudibranch Montereina on stimulation of the visceral nerve. Showing augmentor effects.

vation. When the tissues that must necessarily be left in connection with the aortic sinus, in order not to sever the ventricular nerves, are properly supported, their contraction does not affect the ventricle. That the prolonged diastolic pause following the augmentation is in reality an after-effect of the stimulation seems to be shown by the tracings reproduced in Fig. 13. In *A* the stimulation is purposely continued till the reappearance of the rhythm, and by comparing this tracing with Fig. 12, it will be seen that the appearance of

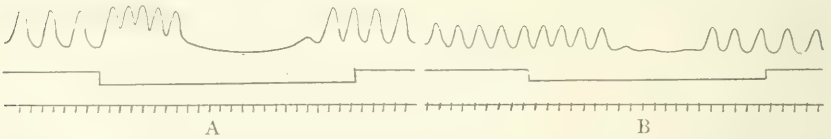


FIGURE 13. — Tracings from the pulsating ventricle of Montereina on stimulation of the visceral nerve. Showing primary augmentation followed by an apparent inhibition Time in seconds.

the diastolic pause, and the reappearance of the rhythm, follow the same course, whether the stimulation is continued or not. That is to say, at the end of the accelerator effects the ventricle does not appear to be influenced by the stimulation of the nerve. The cardio-accelerator nervous mechanism of this mollusc thus appears to fatigue or fail very quickly when excited by electrical stimulation. In some preparations this rapid failure of the nervous mechanism is more marked than in others, and may be strikingly illustrated by using a strength of the interrupted current just sufficient to affect the heart, as is shown in Fig. 13 B. In this record there is only a very slight augmentation of the first three beats following the stimulation of the

nerve, nevertheless, at the end of the fourth beat, the ventricle remains in nearly complete diastole for the space of time required for three beats. The acceleration prior to this prolonged diastolic pause is so slight that it would hardly be discernible on direct observation, in which case the arrest of the rhythm would appear to be due to nervous inhibition. The tracings show, however, that the apparent inhibition is invariably preceded by accelerator effects.

The experiments on the cardiac nerves of *Triopha carpenteri* are attended with greater difficulties on account of the small size of the animal, which prevents direct graphic records being obtained from the ventricle, and renders difficult the isolation of the visceral nerve for the stimulation. In the largest specimens available, the distance from the brain to the heart measured about 2 cm., so that the nerve had to be stimulated at a point only about 1.5 cm. distant from the ventricle, which made errors from the escape of the electrical current directly on to heart more liable. But after taking all precautions against escape of the current, and seeing too that the heart is not interfered with by the pressure of adjacent parts, the reaction of the heart on stimulation of the cardiac nerve point to *the presence of both cardio-accelerator and cardio-inhibitory fibres in this nerve.* This applies to the ventricle in particular. The movements of the auricle cannot be studied satisfactorily when the heart is intact, because every beat of the ventricle displaces the auricle so that it becomes difficult to tell which are active and which are passive movements; and separating the auricle from the ventricle causes so great injury to the auricle that its rhythm usually ceases for good. The observations were, therefore, chiefly confined to the ventricle separated from the auricle by cross-section of the latter near the auriculo-ventricular junction.

The accelerator effect on the ventricle of stimulation of the nerve is very similar to that described for *Montereina*, though somewhat less pronounced. But frequently arrest of the ventricle in diastole followed immediately on sending the current through the nerve, and the cessation of the stimulation was followed by an improved rhythm, or the beats reappeared before the cessation of the stimulation if a weak strength of the stimulus was used, or if the stimulation was long continued. In some records the prolonged diastolic pause is preceded by a brief acceleration of the rhythm, just as in *Montereina*, but the appearance of the pause is dependent on the continuation of the stimulation, and is not an after-effect of

the stimulation. These results in *Triopha* showing the presence of both accelerator and inhibitory nerves to the heart are of peculiar interest, as it is the first indication of a cardio-inhibitory nervous mechanism in the gasteropods so far examined.

Discussion of the general results is deferred to the next paper, which will include the work on the cardiac nerves of the pulmonate and the cephalopod molluscs.

THE INFLUENCE OF ALKALOIDS AND ALKALOIDAL SALTS UPON CATALYSIS.

By ORVILLE H. BROWN and C. HUGH NEILSON.

[*From the Physiological Department of the St. Louis University.*]

ELECTROLYTES influence catalytic and enzymatic activities. This has been shown by numerous observers. Among the earlier workers along this line should be mentioned Oppenheimer,[1] Kubel,[2] Dumas,[3] Grützner,[4] Podolinski,[5] Lintner,[6] Kjeldahl,[7] and Jacobson.[8] The results of these observers need not be given here, as their work was done previous to the Arrhenius theory of dissociation, and consequently was not interpreted according to the ideas of recent chemistry. The later workers have attempted not only to ascertain the influence of the anion and the cation, but to explain the reason for such influence upon the catalytic or enzymatic processes. Cole,[9] working with amylolytic ferments, came to the conclusion that in general cations have a depressing power and anions a stimulating power upon fermentative processes. Neilson and Brown,[10] working with finely divided platinum and organ extracts, came independently to practically the same conclusion as Cole. Kastle and Loevenhart,[11] however, working with finely divided metals as catalytic

[1] Oppenheimer: Die Fermente und ihre Wirkungen, 1900, p. 40.

[2] Kubel: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 276.

[3] Dumas: Comptes rendus, 1872, lxxv, p. 295 (reference from Oppenheimer's Die Fermente und ihre Wirkungen, p. 40).

[4] Grützner: Archiv für die gesammte Physiologie, 1876, xii, p. 285.

[5] Podolinski: Beitrag zur Kenntniss der Pancreas-Eiweissverdauung, Dissertation, Breslau. (From Oppenheimer's Die Fermente.)

[6] Lintner: Journal für praktische Chemie, 1887, xxxvi, p. 841.

[7] Kjeldahl: Comptes rendus des trauvaux du laboratoire de Carleberg, 1879. (reference from Effront's Enzymes and their application).

[8] Jacobson: Zeitschrift für physiologische Chemie, 1892, xvi, p. 340.

[9] Cole: Journal of physiology, 1903, xxx, p. 202.

[10] Neilson and Brown: This journal, 1904, x, vi, p. 335.

[11] Kastle and Loevenhart: Science, 1904, xix, p. 630.

agents, came to the conclusion that ions, as such, have no influence upon catalysis. McGuigan,[1] working with diastatic ferments, came to the conclusion that the "inhibiting power of any salt is inversely proportional to the sum of the solution tensions of its ions, or to the decomposition tension of the salt."

Alkaloids form salts with acids in the same way as ammonia forms salts with acids, that is, by retaining the hydrogen instead of displacing it, as is done when metals unite with acids. However, it seemed to us probable that, as is the case with ammonia, the alkaloidal salts may influence catalysis in a way analogous to the ordinary electrolyte. The object of this research is to determine the action of strychnin and caffein and their salts upon the hydrolysis of dioxide of hydrogen by platinum black and a water extract of kidney or pancreas.

The pharmacological literature contains numerous accounts of researches upon enzymatic activities as influenced by different alkaloids. The results as reported by the different workers are quite inconsistent. As seen by our work this is to be expected, since the observers disregarded the nature of the acid with which the alkaloid was combined.

Popoff[2] has shown that the production of marsh gas from swamp mud is slightly increased by strychnin nitrate. Nasse[3] found that the different alkaloids and alkaloidal salts act differently on the different enzymes. He found that strychnin inhibits the inversion of sugar by diastase, while morphin acetate, veratrin sulphate, and curare accelerate this action; caffein is without effect; veratrin sulphate inhibits the action of ptyalin on starch paste, while the others mentioned above accelerate this action. Nasse also says that the decomposition of hydrogen peroxide into water and oxygen by muscle substance is more inhibited by pure strychnin than by morphin. Wolberg[4] found that in the digestion of fibrin by pepsin, quinin sulphate accelerated the action, while morphin hydrochlorate, narcotin, veratrin, and digitalin inhibited this action. Köhler[5] says that the alkaloids in general have no effect on the decomposition of hydrogen peroxide by the blood.

[1] McGuigan: This journal, 1904, x, vii, p. 444.

[2] Popoff: Archiv für die gesammte Physiologie, 1875, x, p. 132.

[3] Nasse: Archiv für die gesammte Physiologie, 1875, xi, p. 138.

[4] Wolberg: Archiv für die gesammte Physiologie, 1880, xii, p. 291.

[5] Köhler: Archiv für experimentelle Pathologie und Pharmacologie, 1873, i, p. 381.

Methods.

The platinum black used in these experiments was placed in distilled water, and thoroughly stirred at the time of removal. Fresh beef kidney was minced and ground in a mortar with sand; distilled water was added, and the extract was filtered through cheese cloth, and later through filter paper. The extract was always freshly made be-

TABLE I.

Platinum Black.

	1 in 100		1 in 500		1 in 1000		1 in 1500		1 in 2000	
	Cubic centimetres of oxygen given off in									
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Water	13	27								
Strychnin nitrate . . .	2	5	4	8	5	15	12	26	12	28
" hydrochlorate	4	10	7	16	11	24	13	25	13	20
" sulphate . .	8	17	9	21	14	28	17	32	15	29
" phosphate .	8	22	10	24	13	27	13	26	14	27
" arsenate . .	8	18	13	27	12	26	14	27	13	26
" acetate . .	1	24	14	30	14	28	13	28	12	28
" salicylate . .	. .	. .	11	25	13	27	14	26	14	27
" citrate . . .	10	21	12	26	15	30	16	32	13	27
" valerianate .	12	26	13	27	13	28	12	27	13	28
" purum . . .	12	27	13	27	12	28	13	27	13	28

fore each using. The proper concentration of the extract was obtained by diluting with distilled water. 25 c.c. of the solution to be tested were placed in each of two large mouthed bottles of 200 c.c. capacity. To each bottle was then added 5 c.c. of the platinum black solution or the kidney extract, and 5 c.c. of perfectly fresh hydrogen dioxide. To insure accuracy in the readings, the peroxide of hydrogen was added to the bottles simultaneously by pipettes through a second hole in each of the stoppers. These holes were immediately and simul-

taneously closed by means of glass rods. To insure free liberation of the gas, the bottles, sitting in holes in a block of wood, were shaken by hand. The gas was measured in eudiometer tubes, which were connected to the bottles by means of glass tubes. As a control, distilled water was used instead of the alkaloidal solution to be tested. At the end of two and five minutes in case of the kidney extract, and one and two minutes in case of the platinum black, the amount of

TABLE II.

KIDNEY EXTRACT.

Solutions used.	$\frac{m}{100}$		$\frac{m}{300}$		$\frac{m}{3000}$		$\frac{m}{10000}$	
	Cubic centimetres given off in							
	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.
Water	9	36						
Strychnin nitrate	2	15	4	20	5	25	6	30
" hydrochlorate	8	36	8	38	9	40	8	37
" sulphate	18	56	10	35	10	42	9	38
" phosphate	10	38	10	39	9	36	8	36
" arsenate	20	58	11	44	10	43	10	37
" acetate	35	70	29	58	13	41	9	36
" salicylate	..	..	25	60	10	46	8	35
" citrate	12	43	13	40	11	47	8	38
" valerianate	24	49	28	60	17	55	15	50
" purum	..	..	11	42	8	36	9	37

oxygen collected was recorded. As the results of the controls did not vary more than 1 c.c., no error can be ascribed to the method employed.

I. **The action of the different salts of strychnin on the decomposition of hydrogen peroxide by platinum black and by a watery extract of kidney.** — From Table I it will be noticed that in the decomposition of hydrogen peroxide by platinum black in the presence of all concentrations of strychnin nitrate except in the $\frac{m}{10000}$ and those weaker,

the amount of oxygen given off is much less than the amount from the control. The amount of oxygen is least in the $\frac{m}{100}$ solution and increases with each dilution until the $\frac{m}{25000}$ concentration is reached, where the amount is practically the same as where distilled water was used. The hydrochlorate retards less than the nitrate, and each dilution of the stronger solutions allows more splitting of the hydrogen dioxide until the $\frac{m}{10000}$, where, as in the nitrate, the results are practically the same as the control. The sulphate and phosphate retard in the stronger concentrations and allow approximately normal action in the $\frac{m}{2500}$, and show a slight stimulation in the weaker solutions. The arsenate produces no effect, except a slight inhibition in the $\frac{m}{100}$, and possibly a weak stimulation in the $\frac{m}{25000}$ concentration. The acetate, salicylate, citrate, and valerianate do not inhibit the catalysis in any of the concentrations except possibly a very little in case of the $\frac{m}{100}$ citrate.

From Table II it will be seen that the decomposition of hydrogen dioxide by a watery extract of kidney was greatly inhibited by the strychnin nitrate in all except the very weak concentrations. The hydrochlorate in all concentrations allows practically normal action. The sulphate stimulates in the stronger concentrations, and allows normal action in the $\frac{m}{10000}$. The phosphate, arsenate, acetate, salicylate, and valerianate greatly increases the amount of oxygen given off. The amount of stimulation decreases with each dilution until about the $\frac{m}{10000}$, where practically normal action takes place in all except the valerianate, which still stimulates.

II. The action of caffein and its salts on the decomposition of hydrogen peroxide by platinum black and by a watery extract of pancreas. — Tables III and IV show the salts, the concentrations used, and the results obtained.

From Table III it will be observed that in the decomposition of hydrogen peroxide by platinum black, the amount of oxygen given off is greatly decreased by the stronger concentrations of caffein bromide. The amount of oxygen increases with each dilution until the $\frac{m}{25000}$ concentration is reached, where practically normal action was allowed. The hydrochlorate inhibits much less than the bromide in the stronger solutions, and like the bromide, the inhibitory power decreases with each dilution until the $\frac{m}{25000}$ concentration is reached, where the action is about the same as that of distilled water. The other salts and caffein purum have very little effect, except in the strong concentrations, where there is usually a slight inhibition.

From Table IV it will be noticed in the decomposition of the peroxide by a watery extract of kidney, that the pure alkaloid in the stronger solutions has a slight stimulating action. All of the salts in the strong solutions have a marked inhibitory effect. This de-

TABLE III.

PLATINUM BLACK.

Solutions used.	$\frac{m}{100}$		$\frac{m}{500}$		$\frac{m}{2500}$		$\frac{m}{10000}$		$\frac{m}{25000}$	
	Cubic centimetres of oxygen given off in									
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Water	13	27								
Caffein (purum) . . .	13	28	12	27	14	28	13	26	13	27
" nitrate . . .	10	22	12	24	13	26	13	28	12	27
" bromide . . .	1	3	2	5	5	11	9	21	12	26
" hydrochlorate .	5	14	8	20	11	24	12	25	13	26
" sulphate . . .	11	23	12	24	12	26	13	27	14	28
" salicylate . .	12	25	13	26	14	27	12	27	13	28
" arsenate . . .	11	25	12	26	13	27	13	27	14	27
" benzoate . . .	13	26	12	27	13	28	12	28	13	27
" citrate . . .	9	21	11	25	12	26	13	27	13	27
" valerianate . .	11	25	11	26	13	27	14	27	13	28

creases with each dilution, and nearly normal action is allowed in the $\frac{m}{10000}$ concentration.

The action of the salts of strychnin and caffein upon the splitting of hydrogen peroxide when kidney extract is used, is very different from the action when platinum black is used. The salts of strychnin which inhibit the platinum black catalysis, inhibit also the catalysis by kidney extract, but to a very much less degree. The salts of strychnin, which show little or no inhibition in case of the platinum black, stimulate in case of the kidney extract. The most marked stimulation took place with the salicylate, acetate, and valerianate.

The salts of caffein inhibit in every case the catalysis by platinum black much more than that by pancreas extract.

The inhibitory effect of the caffein salts may be due to the fact that they are acid in reaction, as it is well known that acids have an inhibitory action on the catalysis. This seems very unlikely to us,

TABLE IV.

KIDNEY EXTRACT.

	$\frac{m}{100}$		$\frac{m}{300}$		$\frac{m}{2500}$		$\frac{m}{10000}$		$\frac{m}{25000}$	
	Cubic centimetres of oxygen given off in									
	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.	1 min.	2 min.
Water	9	36								
Caffein (purum) . . .	10	40	10	39	9	38	9	37	10	36
" nitrate . . .	0	0	0	0	1	6	5	18	7	25
" bromide . . .	0	1	1	5	5	25	7	30	8	32
" hydrochlorate .	0	2	1	6	7	30	8	35	9	36
" sulphate . . .	0	1	0	2	2	7	5	26	9	36
" salicylate . .	0	2	0	6	6	30	8	35	9	37
" arsenate . . .	1	2	3	7	6	32	7	33	9	36
" benzoate . . .	1	7	4	15	7	25	8	35	9	37
" citrate . . .	0	1	2	7	5	17	8	34	9	36
" valerianate . .	2	12	6	26	8	34	9	36	9	37

as the acidity is very slight. At any rate this cannot account for the marked inhibition in the case of the hydrobromate, as this salt is not more acid than the others.

The strychnin salts in the concentrations used, with the possible exception of the citrate, are neutral in reaction.

III. The action of the alkaloidal salts and metallic salts upon the splitting of hydrogen peroxide into water and oxygen by platinum black, and by a watery extract of kidney. — The methods of work for obtaining the results of Table V, selected from a previous research,[1]

[1] NEILSON and BROWN: This journal, 1904, x, p. 338.

were essentially the same as those employed in obtaining the results for Tables II and IV, with the exception that in the former pancreas extract was used, while in the latter kidney extract was used. This, however, is unimportant, as pancreas extract was also used with the alkaloids and their salts, and the results were practically identical with those obtained when kidney extract was employed. A table giving the results of the action of the metallic salts upon the split-

TABLE V.

Solutions used.	$\frac{n}{1}$ sol.		$\frac{n}{8}$ sol.		$\frac{n}{64}$ sol.		$\frac{n}{512}$ sol.	
	Cubic centimetres of oxygen set free in							
	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.	2 min.	5 min.
Distilled water	14	33						
Sodium chloride	0½	2	1	3	7	15	14	33
" bromide	2	4	4	8	9	18	13	31
" nitrate	0	0	0	0¼	0	0½	4	7
" sulphate	8	14	11	24	12	25	14	32
" phosphate . . .	..	..	40	70	55	..	20	35
" acetate	..	..	26	53	30	55	25	48
" valerianate . . .	24	40	42	60	39	65	24	50
" citrate	29	37	42	70	45	75	14	38

ting of peroxide of hydrogen by platinum black need not be given, as the results were very similar to those obtained where organ extracts were used.

By comparison of the tables, it will be seen that the salts of hydrochloric, hydrobromic, and nitric acids, whether the base is an alkaloid or a metal, invariably have a retarding effect upon the catalysis. The sulphate of the metal and the hydrosulphate of the alkaloid are uniformly ineffective except in concentrated solutions, where there is depression in case of the metal. The sodium salt of phosphoric acid stimulates considerably, while the alkaloidal salt is comparatively inactive. This is probably due to the fact that the sodium phosphate is alkaline in reaction, even in the concentrations used. The salts of

acetic, valerianic, and citric acids, both metallic and alkaloidal, invariably increase the amount of the catalysis. It will, however, be noticed that the alkaloidal salts are active in much greater dilutions than the metallic salts, and that the alkaloidal salts are less effective upon the catalysis by platinum black than upon that by organ extracts.

It should be noted that the alkaloids were used in fractions of one gram molecular concentrations, so as to have the amount of the uncombined alkaloid in each solution bear exact relations to the concentration. That is, an $\frac{m}{100}$ solution of strychnin hydrochlorate contains exactly the same amount of uncombined alkaloid as an $\frac{m}{100}$ solution of strychnin hydrocitrate.

From the results of the preceding experiments, and those of a former research,[1] it is seen that there is a relation between the action of salts of metals and those of alkaloids upon the catalysis of hydrogen dioxide into water and oxygen by platinum black and organ extracts. It seems that this supports the idea advanced by Dr. A. P. Mathews,[2] that there is a probable analogy between the chemical action of certain inorganic salts and certain organic substances upon protoplasm.

The strychnin salts in $\frac{m}{10000}$ concentrations were injected into frogs in small amounts, and the work thus far shows no such variations as in case of the catalysis. The time of development and the character of the tetanus were not appreciably affected. However it may be said that it is difficult to obtain frogs of exactly the same weight, and difficult to inject and keep retained exact amounts of the solutions. Whether there might be difference in other actions, as, for instance, blood pressure, has not yet been determined; further work is being done to determine this point.

[1] Neilson and Brown: This journal, 1904, x, p. 335.

[2] Mathews: This journal, 1905, xii, v, p. 419.

THE PRECIPITATION LIMITS WITH AMMONIUM SULPHATE OF SOME VEGETABLE PROTEINS. SECOND PAPER.

By THOMAS B. OSBORNE and ISAAC F. HARRIS.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

IN a former paper on the precipitation limits with ammonium sulphate of some vegetable proteins,[1] we found that in some cases after the degree of saturation had reached a point at which practically all of the protein substance under examination had been precipitated, a small proportion still remained in solution which could be completely removed only by raising the degree of saturation to a very considerable extent. Thus corylin, the globulin of the filbert, was first precipitated when 10 c.c. of its solution contained ammonium sulphate equivalent to 3.7 c.c. of a saturated solution of this salt, and nearly all of it was precipitated when the concentration was raised to one corresponding to a content of 5.3 c.c. On further raising the concentration, it was found that traces of protein still remained in solution until the concentration in ammonium sulphate was made equal to 6.6 c.c.

Whether or not this substance, which had a final limit of precipitation so much higher than that of the greater part of the preparation, was another protein which we had failed to separate, or an alteration product of the corylin which had formed in small quantity on drying, required a further study, which we have since made. The unexpected results of this investigation deserve careful consideration, for they appear to show that the use of this ammonium sulphate method for the detection of different protein substances in an extract cannot be relied on to the extent that has been customary during the past few years.

In order to determine whether the limits of precipitation of a preparation were the same after it had been washed and dried as they

[1] Osborne and Harris: Journal American Chemical Society, 1903, xxv, p. 837.

were at the time it was separated from the extract, we treated 2100 gm. of fine, oil-free meal of filberts with 10 per cent sodium chloride solution, filtered the extract clear, and dialyzed it for seven days. The resulting precipitate, which, according to our former experience, consisted of corylin, was filtered out and dissolved in one tenth saturated ammonium sulphate solution. To the perfectly clear solution which resulted we added saturated ammonium sulphate solution in such quantity that the dissolved salt was equivalent to a mixture of 40 c.c. of the saturated solution and 60 c.c. of water, or, in other words, to four tenths saturation. In doing this, account was taken of the sulphate present in the one tenth saturated solution in which the protein was dissolved.

Since only a very small precipitate was thus produced, it was filtered out and rejected, and the concentration of the solution in sulphate was raised to five tenths. This caused a large precipitate, which soon settled to a coherent layer, from which the solution was decanted clear, and its concentration in sulphate raised to six tenths. A flocculent precipitate was thus produced, which was filtered out. The filtrate contained very little more protein, and was not further examined.

The two fractions which had been separated from the extract, No. 1, between four tenths and five tenths, and No. 2, between five tenths and six tenths saturation, were separately dissolved in 10 per cent sodium chloride solution, and the globulin precipitated by dialysis. The precipitates which had formed after six days were filtered out, washed with water and alcohol, and dried over sulphuric acid in the usual way. No. 1 weighed 57 gm.; No. 2 weighed 14.84 gm. The precipitation limits were then determined, immediately after these preparations had been made, in the way described in our earlier paper, already referred to.

2.5 gm. were dissolved in 50 c.c. of one tenth saturated sulphate solution, filtered clear, and the solution used for duplicate determinations with the following results, which are given in terms of cubic centimetres of saturated ammonium sulphate in 10 c.c. of the solution, allowance being made for the sulphate contained in the one tenth saturated sulphate solution present in the 2 c.c. of protein solution taken, as well as that used to bring the total volume to 10 c.c.

No. 1. 0.4–0.5.

	I.	II.
Lower limit	1.9 c.c.	1.9 c.c.
Upper limit	4.3 c.c.	4.2 c.c.

After keeping this preparation in stoppered bottles for six months, this test was repeated with the following result:

Lower limit	1.9 c.c.	..
Upper limit	4.51 c.c.	..

Under the same conditions, when freshly prepared, No. 2 gave the figures given below:

No. 2. 0.5–0.6.

	I.	II.
Lower limit	2.15 c.c.	..
Upper limit	4.60 c.c.	4.60 c.c.

After keeping for six months:

Lower limit	2.3 c.c.	..
Upper limit	4.5 c.c.	..

From these results it appears that although little if any change occurred on keeping, the precipitation limits of the isolated protein in neither case were the same as before separation, but were very distinctly lower, as well as wider apart, so that the fractions which had remained in solution when the original extract was four tenths saturated with ammonium sulphate were to a large extent precipitated at a concentration below four tenths saturation, after they had been dissolved in sodium chloride solution, precipitated by dialysis, and washed and dried in the usual manner.

It is to be noted also that neither of these fractions had so high an upper limit as the preparation previously examined, and described in our earlier paper, and we therefore conclude that our former preparation was slightly contaminated by proteose-like substances which were largely separated from the preparations here described by the fractional precipitations, and that this higher limit of precipitation was not caused by washing the corylin with alcohol or by drying, as was suggested in our former paper might be the case.

We next applied this method to a quantity of the globulin obtained from the yellow lupin, which, as a previous investigation [1] had shown, consists of two different protein substances. A quantity of the mixed globulin, weighing 600 gm., which had been extracted from the lupin seed by sodium chloride solution, and precipitated by dialysis, was dissolved in one tenth saturated ammonium sulphate solution,

[1] Osborne and Campbell: Journal American Chemical Society, 1897, xix, p. 454.

and to the clear solution which resulted, saturated sulphate solution was added to five tenths saturation. A semi-fluid precipitate, I, separated, which was dissolved in 600 c.c. of one tenth saturated sulphate solution, and 500 c.c. of saturated added, making a very nearly five tenths saturated solution. The precipitate so produced was dissolved in 300 c.c. of one tenth saturated, and re-precipitated by 250 c.c. of saturated sulphate. The substance thus thrown down was again dissolved in 10 per cent sodium chloride brine and its solution dialyzed for several days, yielding Preparation 1.

The solution from which the above substance was separated, when brought to five tenths saturation for the first time, was mixed with enough saturated sulphate solution to make six tenths saturation, and the solution decanted from the precipitate which resulted. The latter, together with the precipitate obtained by raising to six tenths saturation the filtrate from the second precipitation of I at five tenths saturation, was redissolved in 1000 c.c. of one tenth saturated sulphate, and the clear solution brought to five tenths saturation. It remained perfectly clear. The saturation was then raised to six tenths, when a precipitate, II was produced. This was united with a precipitate obtained by raising to six tenths saturation the filtrate from the third precipitation of I at five tenths saturation, and all was then dissolved in 500 c.c. of one tenth saturated sulphate. This solution was precipitated by raising the saturation to six tenths, and the precipitate produced was re-precipitated in the same way. The final product was then dissolved in 10 per cent sodium chloride brine, and the clear solution dialyzed for several days, and the substance which separated on dialysis was washed with water, giving Preparation 2.

The filtrate from the first precipitation of II at six tenths saturation was made seven tenths saturated, and the precipitate that formed, together with that produced by raising to seven tenths the filtrate from the second precipitation of II at six tenths saturation, was dissolved in 500 c.c. of one tenth saturated sulphate solution, and its concentration raised to seven tenths. The resulting precipitate was dissolved in 10 per cent sodium chloride brine and its clear solution dialyzed for several days, yielding Preparation 3, which was washed thoroughly with water. The solutions remaining from the re-precipitations of II and III, from which the protein precipitated at seven tenths saturation had been separated, were united and saturated with ammonium sulphate. The resulting precipitate was dissolved

in sodium chloride brine, and the clear solution dialyzed for some time. The substance deposited was then thoroughly washed with water, giving Preparation 4.

The precipitation limits of these repeatedly precipitated fractions were determined before washing them with alcohol or drying over sulphuric acid. The solutions used contained approximately 5 per cent of the substance dissolved in one tenth saturated sulphate solution. After making these determinations the preparations were washed with alcohol, dried over sulphuric acid, and found to have the weights given below. After keeping in stoppered bottles for six months, the precipitation limits were again determined. The results of these determinations were as follows:

Preparation 1. 0.0–0.5; weight, 30 gm.

	At once.	After six months.
Lower limit	..	3.25 c.c.
Upper limit	5.5 c.c.	5.50 c.c.

Preparation 2. 0.5–0.6; weight, 105 gm.

	At once.	After six months.
Lower limit	3.7 c.c.	3.7 c.c.
Upper limit	5.5 c.c.	5.5 c.c.

Preparation 3. 0.6–0.7; weight, 113 gm.

	At once.	After six months.
Lower limit	3.7 c.c.	3.8 c.c.
Upper limit	6.4 c.c.	6.4 c.c.

Preparation 4. 0.7–1.0; weight, 66 gm.

	At once.	After six months.
Lower limit	4.6 c.c.	4.6 c.c.
Upper limit	7.75 c.c.	8.2 c.c.

In another experiment we extracted 330 gm. of oil-free meal of the Brazil nut (*Bertholetia excelsa*) with 1500 c.c. of one tenth saturated ammonium sulphate solution, filtered the extract, and added to 1000 c.c. of the clear solution 490 c.c. of saturated sulphate, thereby raising it to four tenths saturation. As the solution remained perfectly clear, its saturation was made five tenths by adding 302 c.c. more of the saturated sulphate solution. The precipitate thus produced was filtered out, dissolved in one tenth saturated sulphate solution, and again precipitated at five tenths saturation. The pre-

cipitate which resulted was dissolved in one tenth saturated sulphate, and its clear solution dialyzed, giving Preparation I, consisting wholly of hexagonal crystals, which, when washed with water and alcohol and dried over sulphuric acid, weighed 24.5 gm.

The filtrate from the first precipitation of I, at five tenths saturation, measuring 1600 c.c. was brought to six tenths saturation by adding 400 c. c. of saturated sulphate solution, and the precipitate produced redissolved in one tenth saturated sulphate, and again thrown down at six tenths saturation. The precipitated protein was then dissolved in one tenth saturated sulphate solution, and separated by dialysis.

This gave Preparation II, which consisted of a mixture of imperfectly formed crystals and spheroids, and, when washed and dried in the usual manner, weighed 5.0 gm.

The filtrate from the first precipitation of Preparation II at six tenths was raised to seven tenths saturation, but only an insignificant precipitate resulted. The solution was therefore saturated with sulphate, the precipitate dissolved in water and dialyzed. The substance which was thus separated, Preparation III, when washed and dried, weighed 5 gm. The precipitation limits of these preparations were then determined with the following results:

Preparation I. 0.4–0.5; 24.5 gm.

Lower limit	1.9 c.c.
Upper limit	4.6 c.c.

Most was precipitated between 2.1 c.c. and 4.2 c.c.

Preparation II. 0.5–0.6; 5.0 gm.

Lower limit	2.1 c.c.
Upper limit	6.0 c.c.

Most was precipitated between 2.5 c.c. and 5.5 c.c., while much had separated at 3.0 c.c.

Preparation III. 0.6–1.0; 5.0 gm.

Lower limit	4.1 c.c.
Upper limit	7.5 c.c.

Most was precipitated between 4.8 c.c. and 7.3 c.c., while much separated with 5.5 c.c.

In these results we have a full confirmation of those obtained with corylin, the limits of precipitation of the isolated protein being lower

and wider apart than those at which the protein was first separated. This change is not due to drying or to washing with alcohol, but appears to be in some way connected with the precipitation by dialysis, since in all these experiments the limits of the different fractions were practically constant before they were dissolved in salt solution and precipitated by dialysis. In this connection it is to be noted that a very large loss of proteid occurred in the case of the lupin globulin, since only 314 gm. of the 600 first taken were obtained in the several fractions, although nearly all that could be precipitated by ammonium sulphate was recovered.

The same loss also took place with the hazel nut extract, from which only 83.5 gm. of protein were secured, while that originally present was very much greater.[1]

This investigation shows that fractional precipitation with ammonium sulphate in a seed extract cannot be considered as demonstrating the presence of one or several proteins, since even though the limits of these fractions may differ from each other by a very considerable amount, the limits of the fractions of the isolated protein may be quite the same as one another. It may be urged that this only shows that the protein before separation is not the same as that isolated by the usual processes, but in this connection it is to be observed that our preparation of crude conglutin which had been separated and prepared in the usual way showed the same behavior as the protein extracted directly from the hazel and Brazil nuts.

Although it is evident that we cannot use this method of fractional precipitation with ammonium sulphate for certainly identifying an individual protein, it is nevertheless very useful as a means of separating mixtures of two different proteins.

In order to learn if the separation by ammonium sulphate had yielded products of different composition from those formerly obtained in this laboratory, and also to determine whether the fractions separated at different concentrations of the ammonium sulphate solution differed in composition, we analyzed the products obtained in this investigation, after drying them at 110°, with the following results:

[1] A similar loss of protein takes place in extracts of the flaxseed, according to observations made by one of us. OSBORNE: American Chemical Journal, 1892, xiv, p. 657. This may possibly be due to autolysis.

CORYLIN, FROM THE FILBERT.

Preparation I. 0.4–0.5.		Preparation II. 0.5–0.6.
Carbon	51.44	51.40
Hydrogen	6.94	6.54
Nitrogen	19.07	19.03
Sulphur	0.55	0.55
Oxygen	22.00	22.48
	100.00	100.00

These analyses show no difference in composition between the two fractions which were separated from the extract at different concentrations by the ammonium sulphate, but which, after separation, had very nearly the same limits of precipitation as one another.

These preparations agree closely in composition with that formerly analyzed in this laboratory,[1] the slight differences being doubtless due to the somewhat greater purity of the present preparations.

Conglutins from the Yellow Lupin.

In a former paper from this laboratory [2] we showed that from extracts of the yellow lupin two similar globulins could be obtained by fractional precipitation from their solution in sodium chloride brine. These globulins differed chiefly in their sulphur content. This was

CONGLUTINS FROM THE YELLOW LUPIN.

Preparation.	1. 0.0–0.5	2. 0.5–0.6	3. 0.6–0.7	4. 0.7–1.0
Carbon	51.76	51.74	51.41	49.91
Hydrogen	7.05	6.87	6.86	6.81
Nitrogen	17.57	17.69	17.59	18.40
Sulphur	0.61	0.63	0.81	1.67
Oxygen	23.01	23.07	23.03	23.21
	100.00	100.00	100.00	100.00

[1] Osborne and Campbell: Journal of the American Chemical Society, 1896, xviii, p. 609.

[2] Osborne and Campbell: Journal of the American Chemical Society, 1897, xix, p. 454.

confirmed by the following analyses of the products obtained from the mixed globulin of this seed by fractional precipitation with ammonium sulphate.

The Preparations 1 and 2 have each practically the same composition, and nearly the same as that formerly found for the Conglutin A, as the following figures show:

CONGLUTIN A.

	Average Preparations 1 and 2.	Average former preparations.
Carbon	51.75	50.91
Hydrogen	6.96	6.88
Nitrogen	17.57	17.74
Sulphur	0.62	0.52
Oxygen	23.10	23.95
	100.00	100.00

CONGLUTIN B.

	Preparation 4.	Average former preparations.
Carbon	49.91	49.58
Hydrogen	6.81	6.80
Nitrogen	18.40	18.27
Sulphur	1.67	1.65
Oxygen	23.21	23.70
	100.00	100.00

The slight difference in composition between the preparations made by fractional precipitation from sodium chloride solutions and those made by fractional precipitation by ammonium sulphate are, we think, for the most part due to the better separation obtained by the latter process, which is a more efficient and far less tedious process for the separation of two similar proteins than fractional precipitation from sodium chloride solutions. It is in just such cases as this that Hofmeister's process is especially useful, and constitutes an indispensable means for preparing large quantities of protein in a pure state.

Excelsin from the Brazil Nut.

We have also analyzed the preparations obtained by the ammonium sulphate method from the Brazil nut in order to compare the composition of the two successive fractions from the extract of this seed with one another, as well as with that of the crystalline preparations formerly made in this laboratory by dialyzing sodium chloride solutions of this globulin.[1]

[1] Osborne: American Chemical Journal, 1892, xiv, p. 662.

EXCELSIN FROM THE BRAZIL NUT.

	Preparation I. 0.0–0.5.	Preparation II. 0.5–0.6.	Former preparations.
Carbon	52.48	52.25	52.18
Hydrogen	7.11	6.71	6.92
Nitrogen	18.17	18.49	18.12
Sulphur	22.24	22.55	1.06
Oxygen			21.72
	100.00	100.00	100.00

The Precipitation of Globulins and Albumins by Ammonium Sulphate.

Since the introduction of Hofmeister's method of fractional precipitation of proteids by partial saturation with ammonium sulphate, it appears to have become the custom for physiologists to distinguish the globulins and albumins by means of their limits of precipitation with this salt, it being presumed that groups of proteids are thrown down from their solutions at increasing concentrations of this salt in the order named. Thus those proteids which are precipitated below one half saturation are generally designated globulins, those above one half saturation albumins. This practice has become so general that it seems to be considered by many, if not by most writers on this subject, that this property is sufficient to distinguish these two groups of proteids. The older definitions, that of a globulin as a native protein body insoluble in water, but soluble in dilute saline solutions, and of an albumin as a native protein body soluble in water or dilute saline solutions and coagulable by heat, seem now to be largely replaced by treating the globulins as protein bodies precipitated by half saturation of their solutions with ammonium sulphate, and the albumins as protein bodies precipitated at a higher concentration of this salt. If this latter mode of dividing the proteids into different groups is to prevail, the old ideas respecting the solubilities of the substances to which these designations have been attached must be abandoned by those who have to do with the vegetable proteins; for we have found that the Conglutin B is precipitated above seven tenths saturation, the globulin of the cottonseed from forty-six hundredths to sixty hundredths; legumin, fifty-four hundredths to seventy-five hundredths; phaseolin, sixty-four hundredths to eighty-eight hundredths, while a part of the corylin and excelsin, as described in this paper, were precipitated at from five tenths to six tenths saturation. All of these proteids are well characterized

globulins, according to the older use of this name, being insoluble in water, but readily soluble in dilute saline solutions, from which they are precipitated by dilution or by dialysis. Furthermore, we have found that leucosin is precipitated almost completely from the aqueous extract of the wheat germ at five tenths saturation. This is one of the few protein bodies as yet obtained from plants which conforms to the older definition of an albumin.

In confirmation of these statements we will add that recently we have obtained a fractional precipitate by freeing the sodium chloride extract of the castor-bean from the greater part of the globulin which it contained, adding ammonium sulphate to forty-five hundredths saturation, dissolving the precipitate in water, re-precipitating at one half saturation, again dissolving and re-precipitating at one third saturation. The aqueous solution of this last precipitate on dialyzing for eleven days in water yielded 5.74 gm. of protein soluble in salt solutions, and the filtrate from this globulin, when evaporated to dryness at 50°, left a residue weighing 12 gm., which was *completely* soluble in distilled water and contained 73 per cent of coagulable proteid, the remainder being proteose. Here we see that even after three precipitations at and below half saturation, the last being only one third saturation, we obtained a precipitate containing, according to the older definitions, albumin, globulin, and proteose, all in considerable proportion. In view of these results, all who wish to retain the ideas formerly attaching to the convenient, though undoubtedly unscientifically established, groups of globulins and albumins, will have to abandon the use of partial saturation with ammonium sulphate as a means of distinguishing these groups in vegetable extracts.

The "*pseudo-globulin*" of serum, which is a "*globulin*" soluble in water alone, would seem to be a case, among animal proteids, where this group distinction also fails to apply.

Conclusions.

1. Hofmeister's method of fractional precipitation of proteins from their solutions by ammonium sulphate affords, in most cases, a valuable and ready means for the separation of such substances when associated in the same solution.

2. The precipitation limits are *not* characteristic for each individual protein substance, as is commonly assumed, but appear to depend on the conditions existing in the solution at the time of precipitation.

3. In several experiments the two products obtained, the one by precipitating a large part of the dissolved protein at a definite degree of saturation, and the other obtained from its filtrate at one tenth higher saturation, after separation from solution by dialysis, were found to have the same limits of precipitation as one another, which limits were lower and wider apart than those between which the separation was first made. The products were also alike in composition and properties.

4. Vegetable globulins cannot be distinguished from vegetable albumins by means of their precipitation limits with ammonium sulphate, since many of these globulins are not precipitated until the concentration in ammonium sulphate is raised well above one half saturation, while leucosin, the best characterized albumin of vegetable origin now known, is almost completely precipitated at one half saturation.

CONTRIBUTION TO OUR KNOWLEDGE OF THE ACTION OF PEPSIN, WITH SPECIAL REFERENCE TO ITS QUANTITATIVE ESTIMATION.

By PERCY W. COBB.

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A REASONABLY accurate and simple method for the quantitative estimation of pepsin is necessary in work that is contemplated in this laboratory. The method that seems to answer these requirements is that of Mett. In this method small tubes filled with coagulated egg-white are placed in the solution under examination, the whole kept at body temperature for a definite time (ten to twenty-four hours by various users of the method), and then the length of the digested column measured off.

In using this method the first question that naturally arises is: Is there any definite mathematical relation between the amount of pepsin present and the length of the column digested? It has been shown by several observers, using other methods as well, that the amount of proteid digested is, other conditions being the same, proportional to the square root of the amount of pepsin present. This relation is called the Schütz-Borissow rule.

E. Schütz,[1] who has the credit of first proving this relation, used a solution containing 1 per cent egg-albumin, and precipitated, after digestion, the unchanged albumin, the acid albumin, and all other substances precipitable by ferric acetate with heat. The amount of lævorotation due to the proteids remaining in solution he estimated with the polarimeter, and took this as the measure of the peptic activity.

J. Schütz[2] used a similar egg-albumin solution, precipitated unchanged albumin and acid albumin, estimated the remaining "uncoagulable nitrogen" by the Kjeldahl method, and treated this as the measure of the peptic activity.

[1] Emil Schütz: Zeitschrift für physiologische Chemie, 1885, ix, p. 577.

[2] Julius Schütz: Zeitschrift für physiologische Chemie, 1900, xxx, p. 1.

Both of these observers obtained series of results which correspond well with the Schütz-Borissow rule.

It has also been shown (Borissow,[1] Samojloff,[2] and Nirenstein and Schiff[3]) that this rule holds true when the Mett method is used. J. Schütz gives results showing that in Mett's method the rule holds when the pepsin concentration is forty to fifty times as great as is allowable in his own method. In the latter case he found that if in the course of the experiment about half of the original albumin is used up, the digestion is less than the rule calls for, and he attributes this result to the scarcity of available albumin. In the case of Mett's method, the available albumin is of course a constant quantity.

In the present work Mett's method is used as follows:

Glass tubing of 2.5 to 3 mm. bore is cut into lengths of about 20 cm. These are washed with soap, then with alcohol, and finally with ether, dried, the ends drawn to a capillary and broken off.

The whites of two eggs are beaten to a stiff froth and left covered in a cool place to subside. The resulting liquid, after straining through muslin, is sucked into the tubes, leaving in each tube a space about 1 cm. long. The capillary ends of each tube are fused air-tight as soon as this is done.

A large vessel of water is heated to 95 C., and the tubes placed therein horizontally on wire gauze. The bulb of a thermometer is placed among the tubes and kept as near as possible at 95 C. for fifteen minutes. The flame is then withdrawn and *the tubes left in the water* until the whole is cold. The result of removing the tubes from the hot water is a retraction of the egg-white from one side of the tube for a large part of its length, rendering much of the material useless. When left in the water, and consequently cooled slowly, this takes place as well, but only for a short distance near the ends.

This modification of the method (involving the sealing of the tubes before coagulation) seems, as a result of the increased pressure within the tube on heating, to prevent the formation of air-bubbles in the coagulum, and has the further advantages that it keeps the contents of the tube aseptic and prevents drying.

As to the comparative behavior of old tubes and those freshly coagulated, it can be said that tubes twenty-four hours old gave a somewhat higher reading than others three weeks old, under identi-

[1] Borissow: *Vide* J. Schütz, *Loc. cit.*

[2] Samojloff: *Vide* Pawlow, Glandes digestives, Paris, 1901, p. 38.

[3] Nirenstein and Schiff: Archiv für Verdauungskrankheiten, viii, Heft 6.

cal conditions. This agrees with the statement of Nirenstein and Schiff that tubes less than three days old are digested more rapidly than older ones.

These tubes can be readily cut in the usual way to the needed length (usually 2 cm.), the egg-white breaking off neatly at the fracture.

Certain precautions must be observed in the use of Mett's tubes. The tubes should be put into the solution at once after cutting, as drying of the ends of the proteid column will interfere with the progress of digestion, and the shrinkage by drying will produce an apparent initial column digested. The tubes should be slightly more than just covered by the solution. The reason for this is obvious.

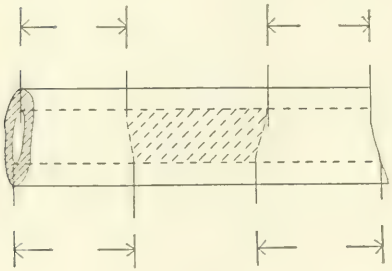


FIGURE 1.

The measurement of the resulting column digested is made with an etched glass millimetre scale, tenths being estimated. The eye is aided by a lens of moderate power. In strongly digestive solutions the ends of the column become somewhat oblique in the course of digestion, and, further, the tubes never break exactly square. To avoid errors from these causes, four measurements are taken from each tube, as indicated in the sketch, the two at the same end always at diametrically opposite points, and measured from the corresponding point on the inside edge of the fractured surface of the glass. Two tubes are usually used, which give eight figures, each estimated to within a tenth of a millimetre. When the average is struck, it is carried out a decimal place further, giving the result in millimetres and hundredths. This result then represents the average length of the empty spaces at the ends of the tubes, and not (as the results are given by some observers) the difference between the length of the original column and that of the column remaining after digestion. The latter would obviously be exactly twice the former.

In this work commercial pepsin is used, sold as "Pepsin, U. S. P. 1 : 3000." When a series is used of uniform strength of hydrochloric acid, the one strongest in pepsin is made by dissolving the pepsin in acid of the required strength, and the others by diluting portions of this with the required amount of the same specimen of acid. Diges-

tion is carried on in small Erlenmeyer flasks of about 70 c.c. capacity, kept corked during the progress of the test.

The following show the relation found between pepsin concentration and column digested:

I. — HCl, $\frac{1}{20}$ normal. Each test 15 c.c., 20 hours at 38°–40° C.

Pepsin	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$ per cent.
Digested	7.75	7.33	5.24	4.16	3.12	2.94 mm.
Calculated[1]	13.00	9.16	6.50	4.58	3.25	2.29 mm.

II. — HCl, $\frac{1}{20}$ normal. Each test 20 c.c., 20 hours at 38° C.

Pepsin	1.00	0.48	0.25	0.09	0.04	0.01	0.001 per cent.
Digested	5.99	5.20	4.12	2.91	2.07	1.11	0.35 mm.
Calculated	10.20	7.06	5.10	3.06	2.04	1.02	0.32 mm.

III. — HCl, $\frac{1}{20}$ normal. Each test 20 c.c., 20 hours at 37½° to 38 C. Tests placed on a rocking platform tilting over and back once a minute.

Pepsin	0.49	0.36	0.25	0.09	0.01 per cent.
Digested	6.28	5.66	5.04	3.35	1.45 mm.
Calculated	7.65	6.56	5.47	3.28	1.09 mm.

IV. — HCl, $\frac{1}{6}$ normal (0.61 per cent). 20 hours.

Pepsin	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$ per cent.
Digested	5.57	4.32	3.24	2.59	1.91	1.24	0.83	0.51 mm.
Calculated	7.13	5.04	3.56	2.52	1.78	1.26	0.89	0.63 mm.

In two of these series the rule of Schütz and Borissow does not hold at all.[2] Its validity (for the lower pepsin-concentrations at least) has been established by various observers using Mett's method. Nirenstein and Schiff have, however, shown that it fails when the pepsin is present in amount more than sufficient to digest 3.9 mm. in twenty-four hours. This would be equivalent to 3.3 mm. in twenty hours, a limit somewhat higher than that indicated by Series II and IV.

[1] The calculated values given are derived from those members of the series which on inspection appear to follow the rule. For instance, in Series II, beginning at the last but one and reading to the left, we have as the pepsin values: 0.01, 0.04, 0.09, 0.25, etc. The columns digested should then be as 1, 2, 3, 5, and so on. They are: 1.11, 2.07, 2.91, 4.12. . . . The first three of these are approximately as 1, 2, and 3. These three are therefore used as the basis of calculation; their sum divided *pro rata* as 1, 2, and 3, and the other values of the series calculated from these by the rule.

[2] These are I and III. The cause for this seemed to be unequal temperature in different parts of the incubator, a condition which was later remedied.

One thing which inspection of the foregoing results will show to be constant, and which is evident to the eye in a glance at Fig. 2, is this: for the higher pepsin-concentrations the observed column digested tends to fall below its theoretical value, more and more as the strength of pepsin is increased.

There are several causes depending on the conditions of the method which may account for this difference. When a proteid is dissolved

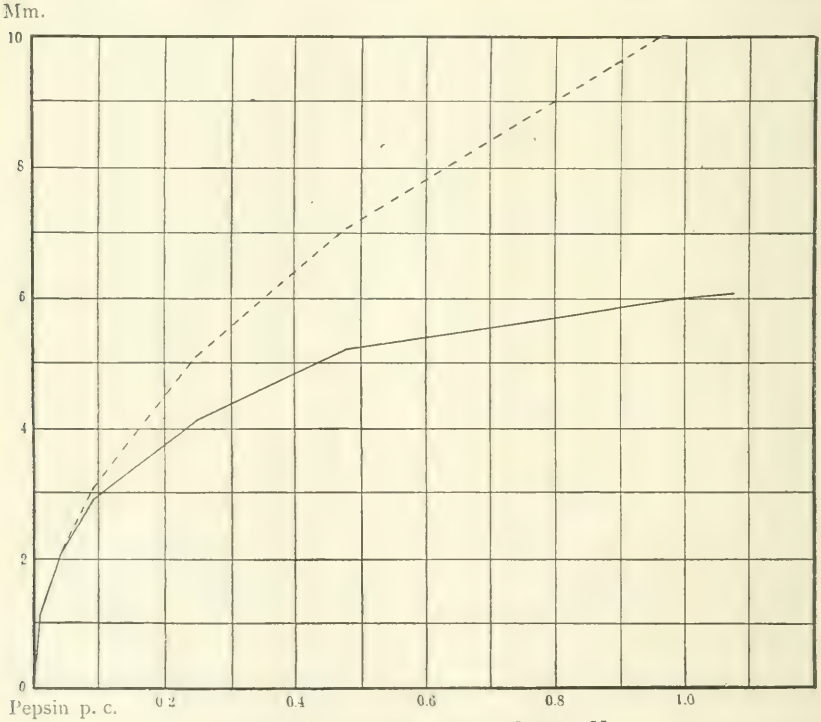


FIGURE 2. REPRESENTING SERIES II.

in a pepsin-hydrochloric-acid solution, (a) proteids of known inhibitory power are formed, and (b) hydrochloric acid is caused to disappear as such, which (in certain cases at least) will diminish the activity of the solution. It can be seen how in Mett's method this might result in two ways: (1) when digestion had proceeded to some depth in the tube, there would be increased difficulty in passage to and from the seat of activity of the fresh and exhausted solution respectively. In this case the disappearance of the proteid column would become slower as its end receded from the end of the tube, and (2) when, as in solutions of higher activity, a longer column is dissolved, the solution at

large becomes exhausted. This would also result in diminished rapidity of digestion as the process proceeded, but can be differentiated from the preceding factor by appropriate methods which will be discussed; and since both of these considerations presuppose a longer column digested, there could never result a smaller column digested (from these causes alone) with a greater pepsin concentration if other conditions were alike.[1]

The first of these points seems to be cleared up by the two following experiments. In V this was done: two Mett's tubes were put into a quantity of pepsin solution, digested for a time (Period 1), measured and returned to the solution with two fresh tubes, digestion allowed to proceed for another period (Period 2), after which all of the tubes were measured and returned with two new ones; and so on, retaining all the tubes until the end of the experiment.

V.—

End of Period No.	1	2	3	4	
Total time, hours	0	21½	43	60	90
Digested in tubes (a)	0.00	2.41	4.51	7.16	9.85
" " " (b)	..	0.00	2.12	4.76	7.38
" " " (c)	..	..	0.00	2.51	5.26
" " " (d)	..	..	..	0.00	2.77

Comparing the amounts digested in tubes at different depths during the same periods (*i. e.*, with other conditions identical) gives:

At depth about	0 to 2½.	2½ to 5.	5 to 7½.	7½ to 10.
Period 2	2.12	2.10	..	..
3	2.51	2.64	2.65	..
4	2.77	2.75	2.62	2.69

Averages of these are:

2, 3, and 4	2.47	2.50	..	..
3 and 4	2.64	2.69	2.63	..

In VI the original tubes were returned and a fresh control set introduced at the end of each period; all the tubes being measured and

[1] NIRENSTEIN and SCHIFF, by using two series of tests, digesting one about half as long a time as the other, obtained results in the former (of course) smaller than in the latter; but each member of the latter series gave a result that bore a constant ratio to that of the corresponding test of the former series. Further, the series of smaller results showed the same deviation from the rule, beginning at the same pepsin concentration.

This speaks against the idea that this discrepancy can be due to the depth of the tube or to the exhaustion of the solution.

the old controls rejected each time. The solution was changed at the beginning of each period; the fresh solution being of exactly the same composition as the original.

VI. —

Total time, hours . .	24	49	$72\frac{3}{4}$
Total depth	2.14	4.61	7.04
Controls	2.25	2.36	2.42

Comparing the amount of digestion in the original set, at a depth, with that in the new tubes for the same periods gives:

Control.	Digestion.	At depth of
2.25	2.14	0 to 2.14 mm.
2.36	2.47	2.14 to 4.61 mm.
2.42	2.43	4.61 to 7.04 mm.

If these differences in the rate of digestion thus found in V and VI are sufficient to be considered at all, they show that with tubes of $2\frac{1}{2}$ to 3 mm. bore and in a pepsin solution capable of digesting about 2.5 mm. in twenty-four hours, (1) digestion proceeds somewhat more rapidly at a depth of $2\frac{1}{2}$ to 5 mm. than it does in a new tube from 0 to $2\frac{1}{2}$ mm., and (2) less rapidly again at depths greater than 5 mm., so that the rate from 5 to $7\frac{1}{2}$ mm. is about equal to the rate at which the first $2\frac{1}{2}$ mm. are digested.

The interpretation of the above seems to be that digestion is delayed more and more as the end of the proteid column recedes from the mouth of the tube, and that, further, there is a slight initial delay when digestion starts in a new tube.

At still greater depths than 7 mm. a marked delay can be shown. In VII, two sets of tubes were digested to the respective depths given in the table under the heading 0 hours, then digested in a similar solution in one flask (*i. e.* under identical conditions) for twenty hours, giving the following results:

VII.

	0 hours.	20 hours.	Difference.
Tubes (a)	1.06	7.26	6.20
Tubes (b)	8.26	13.72	5.46

The foregoing experiments were to clear up the possibility of error depending on the depth of the tube, *i. e.*, the distance from the mouth of the tube to the end of the proteid column. This distance being greater, the difficulty of escape of the exhausted solution and its

replacement by fresh or less exhausted solution is greater, and consequently digestion is retarded.

The second point above mentioned is a different matter. It is apparent that with a stronger pepsin solution more egg-white is dissolved. As a result of this, the solution as a whole is (presumably) exhausted to a relatively greater degree and the resulting column digested is relatively diminished. The depth of the tube *per se* has nothing whatever to do with this factor.

Nirenstein and Schiff have shown that for twenty-four hours digestion goes on at a uniform rate, indicating that no exhaustion of the solution takes place in that time. However, by using in separate tests, respectively, one, two, and four tubes, it is found that the tubes with the larger number of companions give always smaller readings:

VIII. — HCl, $\frac{1}{20}$ normal, time 22 hours. (a) Pepsin, 6 : 1000; each test, 10 c.c.

Tubes in test.	Column digested.	Average of
1	6.46	2 tubes.[1]
2	6.21	4 tubes.
4	6.03	4 tubes.

(b) Pepsin, 3 : 10,000; each test, 10 c.c.

1	2.17	2 tubes.
2	2.12	4 tubes.
4	2.07	4 tubes.

In IX, different amounts of coagulated egg-white were added to each test, digestion being then allowed to proceed until the proteid was completely dissolved. The tests were then investigated in the usual way with two Mett's tubes. The coagulum first added was pushed, by means of a glass rod, out of tubes of 2.8 mm. bore and of the aggregate length given in the first row of figures.

IX. — HCl, $\frac{1}{20}$ normal; pepsin about 3 : 10,000; time, 21 hours; temperature, 38° C.

Albumin added . .	0.00	16.0	96.5 mm.
Digestion	2.44	2.40	2.32 mm.

There is a fair agreement here with the results given in VIII. It is to be remembered that aside from the proteid added at the start, there is a certain amount thrown into solution during the test.

The tubes used in VIII and IX were of 2.8 mm. bore, and the volume of each test 10 c.c. In VIII, there was found a difference of 5

[1] Where one and two tubes were used duplicate tests were made.

to nearly 7 per cent, caused by the use of four tubes in place of one.[1]

It might be well to introduce here some results showing the part played by hydrochloric acid in peptic digestion. In the two series following, the hydrochloric acid was varied, keeping the pepsin-value and other conditions alike.

X. — Time, $22\frac{1}{2}$ hours.

HCl	$\frac{n}{36}$	$\frac{n}{18}$		$\frac{n}{12}$	$\frac{n}{9}$	$\frac{n}{6}$
or	0.10	0.20	0.25	0.30	0.41	0.61 per cent.
Pepsin, 0.50 per cent.	2.51	4.82	5.15	5.16	5.05	4.20 mm.
Pepsin, 0.04 per cent.	1.26	2.06	2.00	2.08	1.94	1.31 mm.

Plotting these results with millimetres as ordinates and hydrochloric acid contents as abscissæ, we have the curves shown in Fig. 3.

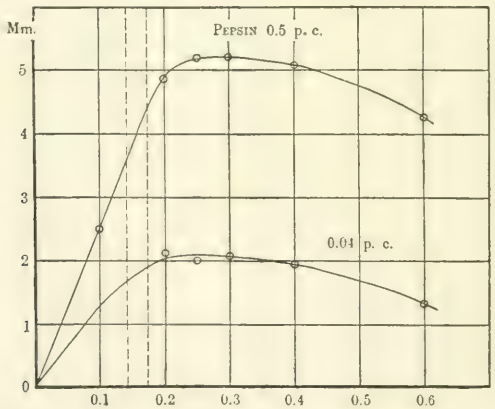


Figure 3.

The limits within which hydrochloric acid may vary without causing change in the column digested are from this 0.25 to 0.3 per cent. In using this method the optimum should be chosen, or preferably

[1] Nirenstein and Schiff state that with tubes of 1 to 2 mm. bore, and using 5 c.c. of gastric juice as a test solution, it makes no difference whether one or three tubes are used.

In a modification of the foregoing experiments done in this laboratory, instead of using a varying number of tubes in the same volume of solution, varying volumes of solution were used with the same number of tubes. The results here were contradictory to the foregoing; in the smaller volumes of solution the columns digested were either equal to or greater than those found in the tests of greater volume. A reasonable explanation of this seems to be that the larger volumes of solution were longer in getting to the temperature of the incubator, and the conditions were thus on the whole less favorable for digestion.

its upper limit, so that as the solution becomes poorer in hydrochloric acid in the course of digestion, the activity of the solution shall not be reduced from this cause.[1]

That the hydrochloric acid loss may be a considerable factor is shown by the following:

XI. — Each test, 10 c.c. HCl, $\frac{n}{20}$ or 1.82 per 1000.

Number of tubes in test . .	0.00	1	2	3
Total column digested . .	0.00	13.70	27.60	39.50 mm.
HCl at end, by titration . .	1.70	1.62	1.52	1.46 per 1000.

In Fig. 3 the two dotted lines correspond to the percentages 1.70 and 1.45 of hydrochloric acid, and it is seen what a difference this change might make. If, however, 0.3 per cent were chosen as the standard strength, this reduction would cause no corresponding change in the result.

The foregoing consideration of the errors inseparable from Mett's method shows that, although worthy of note, they are still inadequate to explain the tremendous differences seen in the case of strong pepsin solutions between the actual result and the one anticipated from the rule of square roots. It has been shown by Nirenstein and Schiff that these differences are still observed when the possibility of inhibition is excluded. This part of their work will shortly be discussed.

That we may introduce inhibitory substances with the pepsin into the solution, whose inhibitory effect diminishes faster than the activity of the pepsin does when diluted with hydrochloric acid of the same strength, is a fact which has been demonstrated by the same observers in the case of gastric contents. They state that it is not uncommon to find that the filtrate from the gastric contents after a test meal has a lower digestive power as it is, than when it is diluted with hydrochloric acid of a strength equal to its own. Diluting in this way 1, 2, 4, 8, etc. times, they find that sometimes the values are increased by the first (twofold) dilution, and that in some cases it is not until the original solution has been diluted eight times that a value equal to or less than that given by the original filtrate is reached.[2]

[1] As pepsins from different species may have different optima this should be first established for the kind of pepsin under examination and the proper standard adopted. This was unfortunately not done in the present work until the greater part of it was completed.

[2] Compare this with XIII, p. 461.

These observers verify their conclusion from such cases (that there are substances in the filtered gastric contents which are inhibitory to peptic activity) in various ways. As part of their evidence, they showed that dextrose, when present in the solution to the extent of 1 per cent, and sodium chloride to the extent of 0.2 per cent will cause a distinct inhibition. These two substances are evidently very apt to be present in gastric contents.

There is one experiment given in detail by these observers which seems to be of especial interest in this connection. Starting with a solution of pepsin and hydrochloric acid of such strength as to digest 7.6 mm. in twenty-four hours, they made two series of tests: (a) by diluting 2, 4, 8, etc. times, with hydrochloric acid of the same strength, and (b) by diluting similarly with the original solution in which the ferment had been destroyed by boiling. These two series gave identical results when examined by Mett's method. It is evident that if in the original solution there were present inhibitory substances, causing the column digested to fall below its calculated value (as it does in Series I, II, III, and IV in the present work) that this would be evident in comparing the equally diluted tests of (a) and (b) respectively, since (a) contains relatively less of the inhibitory substances, and both (a) and (b) contain the same amount of pepsin.

The fact that the two series gave identical results, taken with the fact that both series showed, in those members strongest in pepsin, results below the values given by calculation from the rule, shows then that this deviation is not due to inhibition, but (these observers think) to a fundamental difference in the behavior of the pepsin itself under those conditions.

Inhibition was actually found when the attempt was made to repeat the experiment in this laboratory. The results follow:

XII. — Original solution containing pepsin 1 per cent. Hydrochloric acid 0.28 per cent. Series (a) diluted with hydrochloric acid 0.28 per cent. (b) With some of the original solution boiled and cooled.

No.	Dilution.	(a)	(b)	Ratio a : b.
(1)	1	7.55	7.55	1.000
(2)	4	4.69	4.33	1.083
(3)	16	2.69	2.43	1.154
(4)	64	1.51	1.11	1.361
(5)	256	0.88	0.50	1.760

Here the ratio a : b continues to increase to the very last of the series, the dilution of the inhibitory substances in (a) causing a *relative*

increase in the values. Further, since each test is the fourfold dilution of the one preceding it in the series, its digestive power should (according to the rule) be one-half as great. In 3, 4, and 5 of Series (b) this is seen to hold somewhat better than in the corresponding members of Series (a), the inhibition being uniform in the former case and not in the latter.

Further, commercial pepsin in very concentrated solutions gives much smaller power of digestion than the same solution does when diluted, the acid content remaining the same, as is shown by the following results:

XIII. — Original solution made by adding $\frac{1}{20}$ normal hydrochloric acid, about 10 per cent of its weight of commercial pepsin, the others by diluting this with $\frac{1}{20}$ normal hydrochloric acid.

	Original solution.	Diluted 16 times.	Diluted 256 times.
Gave	2.9	6.4	2.2 mm. digestion.[1]

Ebstein and Grützner[2] (1872) obtained a similar result. They used gastric mucous membrane, which was allowed to digest itself in 80 parts hydrochloric acid 0.15 per cent. The digestive power of this solution was nearly doubled by diluting with four parts of 0.15 per cent hydrochloric acid. Although their method of estimating the digestive activity is open to objection, when compared with more recent methods, they give a series of figures consideration of which leaves at least their comparative value beyond doubt.

It is universally admitted that pharmacopœial pepsin is highly impure from a chemical standpoint. Recently the experiment of dialysing a pepsin solution was tried in this laboratory. A solution was made with 0.25 per cent hydrochloric acid so as to contain about 10 per cent of pepsin, the specimen being commercial pepsin, the same as that used in X and XI. The resulting solution was slightly viscous, and had a deep yellow, turbid appearance. It digested 3.78 mm. egg-white in twenty-four hours.

[1] Two things which might contribute to this paradoxical result are: (1) that the large amount of proteid in the solution may combine with some of the hydrochloric acid, thereby reducing the peptic activity of the solution, and (2) the greater viscosity of the stronger solution would retard circulation and consequently reduce the column digested. That another factor is concerned is however probable, since the solution of the coagulum is not complete in the very strong pepsin solutions, a translucent, jelly-like mass being left in the tube at the end of the test.

[2] Ebstein and Grützner: Archiv für die gesammte Physiologie, 1872, vi, p. 1.

This was dialysed against three times its volume of 0.25 per cent hydrochloric acid for twenty-four hours, at the end of which time much of the yellow color had disappeared and was evident in the dialysate. The solution itself was then capable of digesting 10 mm. in twenty-four hours, this in spite of the fact that some pepsin had escaped into the dialysate, which had at this time a digestive power of 0.92 mm. in twenty-four hours.[1] Further dialysis for twenty-four hours against 15 volumes of fresh 0.25 per cent hydrochloric acid, caused no considerable further increase in the digestive power of the original solution.

Some diffusible substance or substances contained in the pepsin evidently caused an extreme inhibition here, and removal of probably less than three-fourths of these abolished their effect almost entirely.

The artificial gastric juice prepared by dissolving commercial pepsin in weak hydrochloric acid, and still more the filtrate from the gastric contents removed after a test meal, are then very far from being pure solutions of pepsin in weak hydrochloric acid. Two methods of obtaining such a pure, or at least non-inhibitory solution suggest themselves. One is to obtain the gastric juice from the clean and empty stomach of a healthy animal by means of a gastric fistula. This method is described in detail by Schumow-Simanowski,[2] who used such juice in the preparation of what was thought to be pure pepsin.

The other method was tried in this laboratory, and consists in the dialysis of any strong pepsin solution obtained from the gastric mucous membrane against hydrochloric acid of the strength ultimately required, the whole being kept at body temperature during dialysis. In this case the proteid impurities would be digested and pass through the dialyser when they reached the peptone stage, and it is hoped that all of the impurities could be almost wholly eliminated by frequent change of the dialysate. The method has seemed promising, but

[1] It is well to state here that hydrochloric acid alone of the strength used gives absolutely no column digested by Mett's method.

In dialysis under these conditions, not only do the diffusible substances pass out of the pepsin solution, but hydrochloric acid (if this be reduced in amount in the original solution by combination with the proteids) passes in until a concentration is reached equal to that of the dialysate (compare with p. 459, footnote 1).

In this case the dialysate gave on evaporation a fairly large amount of solid matter. On incineration this gave off the odor of burning animal matter, and yielded an insignificant amount of ash.

[2] Schumow-Simanowski: Archiv für experimentelle Pathologie und Pharmacologie, 1894, xxxiii, p. 336.

nothing conclusive has been reached, and it is mentioned here not as a result but only as a suggestion.

In determining the amount of pepsin present in a specimen of unknown strength, the inhibition factor can be eliminated by performing a series of tests, of equal hydrochloric acid concentration, and containing variable known amounts of the substance under examination. The absolute pepsin value can then be deduced from those members of the series which give results consistent with the rule of square roots. Such a series is, for example, the second given in this paper, where the last four tests give consistent results from which an approximate estimate of the absolute amount of pepsin in the original substance (in this case a solid) could be reached.

Bettman and Schroeder[1] have recently devised a method for the estimation of pepsin, the technique of which is as follows: a solution is made containing 1 per cent of desiccated egg-albumin and 0.2 per cent hydrochloric acid; 2 c.c. of this are mixed with 1 c.c. of the liquid under examination (in a two-drachm homœopathic vial), the whole shaken vigorously, and kept at 37° to 40° C. In the absence of pepsin, the froth formed by the agitation is practically permanent. The time of its subsidence is (according to the originators of the method) inversely proportional to the square root of the amount of pepsin present. In support of this conclusion, results are given obtained in series where pepsin solutions of known strength were used, and where the actual time and the time calculated from the above rule agreed with remarkable fidelity.

The present writer has been utterly unable to verify any such relation. With this object in view, three series of observations were made by this method in which pepsin solutions of known strength were used.

The results follow:

XIV. — Albumin solution: Desiccated egg-albumin, 1 gm.; hydrochloric acid, 0.2 per cent; 100 c.c. Pepsin solution in (1) of each series: Commercial pepsin (scales), 0.5 per cent dissolved in hydrochloric acid of 0.2 per cent strength. This was diluted with the required amount of hydrochloric acid (0.2 per cent) to make the pepsin solution for the other tests of the series. In (0) of each series no pepsin was used; in place of the pepsin solution 0.2 per cent hydrochloric acid was added to the test.

[1] Bettman and Schroeder: Medical Record, New York, 1903, Oct. 31.

The tests were made up as follows: 2 c.c. albumin solution were measured into a vial; 1 c.c. of the weakest pepsin solution to be used in the series was added, the vial corked, and given fifty vigorous shakes, and at once put into the water-bath, the time being noted when this is done. The next test was made up in the same way, using the next pepsin solution (in the order of strength), and so on until No. 1 was in the bath. The tests were then all watched, the bath kept as near as possible at 38° C., always between 37° and 40°. When the froth on a test had cleared, leaving only a narrow rim at the junction of the surface of the fluid with the glass, the time was again noted, and the test removed from the bath. The interval between the time the test was put in the bath, and the time at which this end-point was reached, is the time given in the results.

Series a.

Test No. . . .	0	1	2	3	4	5
Rel. pepsin . .	0	1	$\frac{2}{3}$	$\frac{4}{9}$	$\frac{8}{27}$	$\frac{16}{81}$
Time	permanent	7.7	4.7	20.7	25.5	27.5

Series b.

Test No. . . .	0	1	2	3	4	5
Rel. pepsin . .	0	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$
Time	permanent	9.2	19.8	29.6	59.2	83.1

Series c.

Test No. . . .	0	1	2	3	4	5
Rel. pepsin . .	0	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$
Time	permanent	10.2	17.7	30.5	45.9	71.2
Time (calculated)		15.6	22.0	31.2	44.0	62.4

The "time (calculated)" in the last line is inversely as the square root of the amount of pepsin present. The last series is selected for discussion since it is the only one in which the results appear at all to follow any law. In (a) and (b) the results do not apparently give even a clue to any relation between pepsin concentration and time.

In Series (c) the precaution was taken of giving all the utensils a washing in very hot water after the usual cleaning, to destroy any trace of ferment that might modify the results. The ratio of the amount of pepsin in any member of this series to that contained in the next member is $1 : \frac{1}{2}$. The ratio of the times should be (following the rule) inversely as the square roots, or 1 : 1.414. They are actually 1.74, 1.72, 1.50, and 1.55, all of them too high.

If the attempt is made to calculate the relative pepsin-concentrations in any two members of this series by the rule, a large error must

result. For example, in 1 and 5, the times are 10.2 and 71.2, or as 1 to 7. The pepsin values should be, then, inversely as the squares of these, or as 49 to 1. They were actually as 16 to 1.

In conclusion, it can be said in regard to Mett's method:

(1) That with a tube of 2.5 to 3 mm. bore digestion is not retarded by the depth of the tube *per se* at depths of less than 7 mm.

(2) That with such tubes, using 10 c.c. of test solution containing 0.2 per cent hydrochloric acid, a definite decrease in the column digested results from an increase in the number of tubes.

(3) Making all due allowances for errors due to the method itself, pepsin solutions capable of digesting 4 mm. or over in twenty-four hours give results far below those anticipated by the rule of square roots from the amount of pepsin present.

(4) No calculation can be made in any case as to the absolute or relative pepsin values until the question of inhibition is eliminated.

(5) Commercial pepsin sometimes contains considerable amounts of inhibitory substance, evident in solutions of $\frac{1}{64}$ per cent strength.

And in regard to the albumin-froth method of Bettman and Schroeder:

(6) Results obtained by it cannot be justifiably expressed in figures indicative of pepsin-concentration, but only by such expressions as "strong," "very strong," "moderate," etc.

NOTE ON THE PREPARATION OF NUCLEIC ACID.

By HENRY B. SLADE.

HYDROCHLORIC acid does not effect a manageable separation of nucleic acid. In the usual methods of preparing the compound, this is overcome by the use of alcohol. A more convenient method in the preparation of large amounts of the substance is the substitution of magnesium sulphate. In the presence of this salt, hydrochloric acid throws out the nucleic acid in good sized flocks, which rapidly settle so that the mother-liquor can at once be drawn off and the precipitate purified in the usual fashion.

The method in detail consists in a preliminary treatment with caustic soda in the presence of sodium acetate.[1] Fresh brewers' yeast is vigorously stirred with 1.1 per cent of its weight of caustic soda, dissolved in a little water, and two to three times as much crystallized sodium acetate added in bulk. Ordinarily 2.8 pounds of acetate was used to 100 pounds of yeast. The solution is left for twenty-four hours at the ordinary temperature, and then boiled gently for one hour. The hot solution is at once neutralized with glacial acetic acid to faint acid reaction. Magnesium sulphate is added in bulk to the cold filtrate to 5 per cent solution, and then the hydrochloric acid, with constant stirring until a flocculent precipitate forms. The flocks form rapidly, and with excess acid redissolve to a milky liquid. In this case it is necessary to neutralize with caustic soda and to re-precipitate. The presence of 2.5 per cent of strong hydrochloric acid in the total liquor generally effects a complete separation. The yield is 0.5 per cent of the yeast used, and has a very constant phosphorus content of some 7 per cent.

If the method be varied by boiling at once for two hours in the preliminary treatment, instead of allowing the mixture to stand at the ordinary temperature, the amount of nucleic acid precipitated with the hydrochloric acid is greatly reduced, and a nuclein compound, forming an insoluble combination with copper, is formed. The ratio

[1] NEUMANN: Archiv für Physiologie, 1900, p. 159.

of the precipitates with acid and with a copper salt depends upon the extent of the action of the caustic soda. Under varying conditions a series of cleavage products are formed, as Neumann found with thymonucleic acid. The simplest member of this series appears to be a thyminic acid.

Thanks are due to Doctor J. M. Francis, of Detroit, at whose suggestion a study of nuclein compounds was undertaken, for the facilities afforded in this work.

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